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A STATISTICAL STUDY OF THE COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS[1]

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INTRODUCTION

Eriksson and Henning (*3*)[3] and Stakman and Levine (*14*) have shown that the urediniospores of the various biologic forms of *Puccinia graminis* Pers. differ considerably in size. Stakman and Levine also have shown that the urediniospores of any of the biologic forms become appreciably smaller when the rust is grown on fairly resistant host plants or under other extremely unfavorable environmental conditions.

A mathematical analysis of spore measurements in this connection is of considerable value. Pearl and Surface (*7*) say, "It is certain that not only are quantitative methods needed in biology, but also that a far more serious need is for something of the methodological viewpoint." Rosenbaum (*10*) further emphasizes this need by saying "The biometric methods therefore furnish a means of extending the descriptive method and of expressing quantitatively what investigators have heretofore attempted to express qualitatively." This would seem to have a special application in the case of biologic forms, which have always been distinguished on the basis of their physiologic behavior rather than that of their morphologic structure.

The object of this work was to determine by means of statistical studies the morphologic identity, i. e., the limits of variation and the biometric constants for length and width, of the aeciospores, urediniospores, and teliospores of the different biologic forms of stem rust. The studies described in this paper were limited to *Puccinia graminis tritici* Eriksss. and Henn., *P. graminis secalis* Eriksss. and Henn., *P. graminis avenae* Eriksss. and Henn., *P. graminis phleipratensis* (E. and H.) Stak. and Piem., and *P. graminis agrostis* Eriksss. It is intended, however, to extend the study to a number of the more important forms of the wheat stem rust, *P. graminis tritici*, which recently have been discovered.

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[2] The author takes great pleasure in making acknowledgment to Dr. E. C. Stakman and Dr. H. K. Hayes, plant pathologist and plant breeder, respectively, of the Minnesota station, and agent and collaborator, respectively, of the Office of Cereal Investigations, for many valuable suggestions and helpful criticisms.

[3] Reference is made by number (italic) to "Literature cited," p. 567.

EXPERIMENTAL METHODS

The aecial material used for making spore measurements was obtained from several species of barberry (*Berberis* spp.) artificially inoculated with teliospores obtained from wheat, quack grass, oats, and redtop, respectively. The infected plants were collected in the spring of the year at University Farm, St. Paul, Minn. Young, tender leaves of separate barberry plants were inoculated with the teliospores from each of the hosts. Sets of seedlings of wheat, rye, oats, and redtop were inoculated with the mature aeciospores. The infection results thus obtained verified the identity and purity of each of the biologic forms. The aeciospores used for the determination of the spore size of each biologic form were grown under identically the same cultural conditions and on plants of the same barberry species, namely, *Berberis vulgaris* L. In the study of the effect of the host on the dimensions of aeciospores, the cultural conditions were exactly alike, but the barberry species differed in each case. The aeciospores obtained in this manner were first put away as herbarium material and were not measured until several months after maturity. Fifty aeciospores picked out at random from a considerably larger population were measured for both length and width.

Pure strains of urediniospores of each of the biologic forms of *P. graminis* were obtained from specimens of viable uredinial material on grains and grasses, collected at University Farm, St. Paul, Minn., and cultured continuously in the greenhouse on seedlings of different host plants. The measurements in each case were made of healthy urediniospores from the superficial layer of mature uredinia, care being taken to measure spores from uredinia in the same stage of development. In determining the urediniospore dimensions of the individual biologic forms, 200 spores in each population were measured for length and width, respectively, whereas in the study of environmental and host effects 100 spores were measured in every case. For the latter experiment, however, 200 measurements were made of the controls. In the first instance, that is, in the study of the comparative morphology of the urediniospores, the biologic forms concerned were grown on congenial host plants and under uniform and favorable conditions. In the study of relationship of host to urediniospore dimensions, both susceptible and resistant varieties were used, but the environment was maintained uniform and favorable. In the study of the effect of cultural conditions on the size of urediniospores, only one biologic form, *P. graminis tritici*, and one variety of wheat, Haynes Bluestem, were used, the environmental conditions, such as temperature, light, and soil moisture, being interchangeably altered. Control series were conducted simultaneously with the experimental series.

The telial material used in this study was collected at St. Paul, Minn., and kept in the herbarium for nearly a year before the measurements were made. The rust on the common wheat (*Triticum vulgare* Vill.) was *P. graminis tritici;* on the quack grass (*Agropyron repens* (L.) Beauv.) it was *P. graminis secalis;* on the oat (*Avena sativa* L.) it was *P. graminis avenae;* on the timothy (*Phleum pratense* L.) it was *P. graminis phleipratensis;* and on the redtop (*Agrostis alba* L.) it was *P. graminis agrostis.* One hundred teliospores were measured in each test and the statistical results computed from these.

All of the spore measurements were made with the same microscope, carefully calibrated and standardized. The Zeiss screw micrometer was

used throughout the entire period of the work. The illumination for the microscope was derived from artificial light of uniform intensity. The effect of refraction was eliminated as far as possible by mounting the material in drops of water of approximately equal size. It also was attempted to maintain the quantity of water on the slide nearly constant while the measuring of a given population of spores was in progress.

In order to make the measurements of a given group of spores representative and significant, a sufficient number of measurements must necessarily be made. It was found that in the case of aeciospores a minimum of 50 measurements was required. In the case of urediniospores and teliospores at least 100 measurements were necessary. Greater numbers than these did not seem to add materially to the value of the results obtained in the present investigation, although perhaps they might have been useful in some cases. The longitudinal measurements of teliospores were made from the exterior of the apex to the point of attachment of the pedicel, whereas the transverse measurements were made across the septum separating the two cells.

COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS

The following figures show the dimensions of spores of *Puccinia graminis* and *P. phleipratensis*[4] as given by several different workers, who made their studies at different times.

TABLE I.—*Spore dimensions of P. graminis and P. phleipratensis as given by various authors*

Authority.	Range of variation for length and width (in microns).				
	P. graminis.			*P. phleipratensis.*	
	Aeciospores.	Urediniospores.	Teliospores.	Urediniospores.	Teliospores.
Eriksson and Henning (*3, p. 25 and 126*).	[a]14–26	[b]17–40×14–22	[b]35–60×11–22	18–27×15–19	38–52×14–16
McAlpine (*5, p. 121*).....	.....	20–36×14–18	35–63×14–25	.....	.....
Plowright (*8, p. 162*).....	15–25	25–38×15–20	35–65×15–20	.....	.....
Saccardo (*11, v. 7, p. 622, and v. 11, p. 204*).	14–26	24–45×14–21	34–60×12–22	18–27×15–19	35–52×14–16
Sydow (*15, p. 695 and 785*).	14–26	22–42×16–22	35–60×12–22	18–30×15–20	38–52×14–16

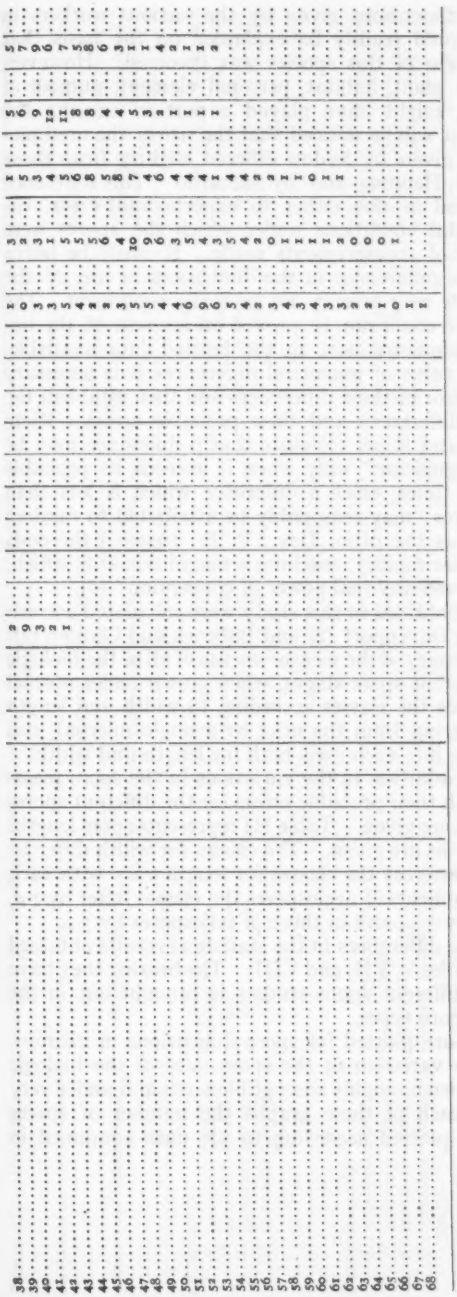
[a] The aeciospore measurements are for range of variation in the diameter of these spores.
[b] These dimensions represent the extremes in range of size of two sets of measurements made by the authors.

[4] *P. graminis phleipratensis* (E. & H.) Stak. and Piem. is cited in this instance as *P. phleipratensis* Eriks. and Henn. because the measurements quoted were obtained from sources in which this biologic form of stem rust was treated as a separate species.

TABLE II.—*Frequency of different sizes of 50 aeciospores, 200 urediniospores, and 100 teliospores in each of certain biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

[Spores.]

Classes according to length and width.	Aeciospores.								Urediniospores.										Teliospores.									
	P. graminis tritici.		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis agrostis.*		*P. graminis tritici.*		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis phlei-pratensis.*		*P. graminis agrostis.*		*P. graminis tritici.*		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis phlei-pratensis.*		*P. graminis agrostis.*	
	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.
Microns.																												
10	....	....	....	2	....	....	....	3	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....	1
11	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	3	....	....	....	....	....	1
12	....	....	1	7	....	1	1	12	....	....	....	....	....	....	....	....	....	....	....	1	....	8	....	3	....	2	....	9
13	....	2	1	19	....	5	2	21	....	....	....	....	....	....	....	1	....	4	....	3	....	10	....	6	....	5	....	14
14	....	4	2	13	....	13	4	11	....	....	....	3	....	....	....	5	....	27	....	6	....	20	....	12	....	13	....	20
15	2	14	6	6	1	22	7	3	....	....	....	10	....	....	....	24	....	52	....	14	....	25	....	22	....	25	....	28
16	2	21	9	3	1	7	14	....	....	2	....	49	....	3	....	46	1	72	....	20	....	18	....	25	....	29	....	14
17	2	7	11	....	6	2	8	....	....	6	....	60	....	7	....	58	1	35	....	27	....	8	....	15	....	17	....	7
18	5	2	8	....	13	....	7	....	....	20	....	47	....	17	2	46	8	10	....	16	....	4	....	10	....	7	....	4
19	7	....	6	....	18	....	4	....	....	44	....	25	....	38	5	16	14	....	....	6	....	3	....	4	....	2	....	2
20	18	....	4	....	9	....	2	....	....	83	2	5	1	70	14	4	23	....	....	6	....	....	....	2	....	....	....	....
21	7	....	1	....	2	....	0	....	....	26	3	1	1	42	16	....	23	....	....	1	....	....	....	1	....	....	....	....
22	4	....	1	....	....	....	1	....	....	15	3	....	3	17	13	....	38	....	....	....	....	....	....	....	....	....	....	....
23	1	....	....	....	....	....	....	....	2	4	11	....	4	6	31	....	28	....	....	....	....	....	....	....	....	....	....	....
24	1	....	....	....	....	....	....	....	3	....	12	....	9	....	31	....	25	....	....	....	....	....	....	....	....	....	....	....
25	1	....	....	....	....	....	....	....	3	....	22	....	14	....	36	....	16	....	....	....	....	....	....	....	....	....	....	....
26	....	....	....	....	....	....	....	....	6	....	24	....	18	....	27	....	11	....	....	....	....	....	....	....	....	....	....	....
27	....	....	....	....	....	....	....	....	9	....	38	....	18	....	15	....	8	....	....	....	....	....	....	....	....	....	1	....
28	....	....	....	....	....	....	....	....	9	....	31	....	39	....	8	....	3	....	....	....	....	....	....	....	....	....	1	....
29	....	....	....	....	....	....	....	....	14	....	24	....	24	....	2	....	1	....	....	....	....	....	....	....	....	....	2	....
30	....	....	....	....	....	....	....	....	16	....	11	....	18	....	....	....	....	....	....	....	1	....	....	....	1	....	1	....
31	....	....	....	....	....	....	....	....	19	....	8	....	13	....	....	....	....	....	....	....	0	....	....	....	0	....	1	....
32	....	....	....	....	....	....	....	....	26	....	3	....	16	....	....	....	....	....	....	....	0	....	....	....	2	....	2	....
33	....	....	....	....	....	....	....	....	15	....	3	....	11	....	....	....	....	....	1	....	0	....	....	....	3	....	4	....
34	....	....	....	....	....	....	....	....	17	....	0	....	5	....	....	....	....	....	1	....	2	....	....	....	2	....	6	....
35	....	....	....	....	....	....	....	....	19	....	2	....	4	....	....	....	....	....	1	....	2	....	....	....	2	....	5	....
36	....	....	....	....	....	....	....	....	12	....	3	....	2	....	....	....	....	....	2	....	2	....	....	....	4	....	5	....
37	....	....	....	....	....	....	....	....	13	....	....	....	....	....	....	....	....	....	3	....	2	....	....	....	5	....	5	....



The differences in the dimensions given by the various workers in the case of *P. graminis* could readily be explained on the basis that more than one biologic form of this species was involved. However, other factors, such as host plant, habitat, personal element, number of measurements made, etc., might have played a part in causing the variation. From Table I, it will be noticed that the size of the urediniospores of timothy rust as given by Sydow differed slightly from those recorded by Eriksson and Henning and by Saccardo. This difference perhaps might be explained as due either to experimental error, variation in the environmental conditions under which the spores were developed, or the number of measurements made. It must be borne in mind, however, that the differences in the dimensions of any of the spores indicated in the table may or may not be really significant. The significance of the difference could not be proved on account of the impossibility of applying a biometric test to the limited data available. In order to determine the value of numerical differences it is necessary to calculate the probable error of these differences and then establish the relationship between the differences and their probable errors. Rietz and Smith (*9*) and Pearl and Miner (*6*) state that a difference may be considered as "certainly significant" only when it exceeds its probable error by more than three times, since a difference which is less than three times its probable error "may reasonably be attributed to random sampling" (*9*), and "that as the ratio, Dev.: P. E., passes 3 the odds against the deviation increase rapidly, reaching a magnitude at 8, which, practically speaking, is beyond any real power of conception" (*6*).

The biometric constants obtained in this study were calculated according to the methods given by Davenport (*2*) and Babcock and Clausen (*1*).

Table II represents a summary of the measurements of all of the biologic forms studied, in their different spore stages, when grown under normal conditions. This table gives the classes according to width and length in microns (each class differing from the next by a single micron), and the number of spores falling into each class both for length and width.

In Tables III and IV, in addition to the variations in the spore measurements, the constants with their probable errors for the different spores of the various biologic forms are given. Table III represents spore lengths while in Table IV spore widths are given. The classes for width differ by one micron (μ) in all cases, whereas the classes for length vary with each spore type. In the measurements of aeciospores the classes differ by 1 μ, in those of urediniospores by 3 μ, and in those of teliospores by 5 μ.

The probable errors of the differences in the statistical results obtained in this study are given in Tables V, VII, and VIII. The bearing they have on the dimensions of the different spore studies is brought out in the discussions of each of the spore types.

In figures 1 to 6, curves are plotted for spore dimensions in microns. These graphs represent the variation in the spore sizes of the biologic forms when the rust was grown on highly susceptible hosts and under very favorable conditions. In each of these graphs the number of spores falling into each class was plotted according to the data contained in Tables III and IV.

TABLE III.—*Variations and constants for length of spores of biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

Spores and biologic forms.	Host plants.	Spore classes according to length.													Total number.	Constants.		
		12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ	25μ	Mean.	Standard deviation.	Coefficient of variability.
Aeciospores:																		
P. graminis tritici....	*Berberis vulgaris*....	.....	.....	.....	2	2	2	5	7	18	7	4	1	1	1	50	19. 72±0. 19	1. 98±0. 13	10. 05±0. 68
P. graminis secalis....	.....do.....	1	1	2	6	9	11	8	6	4	1	1	.....	.....	.....	50	17. 10± . 19	2. 00± . 13	11. 70± . 79
P. graminis avenae....	.....do.....	.....	.....	.....	1	1	6	13	18	9	2	.....	.....	.....	.....	50	18. 62± . 11	1. 20± . 08	6. 44± . 43
P. graminis agrostis..	.....do.....	1	2	4	7	14	8	7	4	2	0	1	.....	.....	.....	50	16. 46± . 18	1. 93± . 13	11. 73± . 79
		15μ		18μ		21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ					
Urediniospores:																		
P. graminis tritici...	*Triticum vulgare*....	.....		.....		.....	8	24	49	58	44	14	3			200	32. 40±0. 19	3. 89±0. 13	12. 00±0. 41
P. graminis secalis....	*Secale cereale*.....	.....		.....		8	45	93	43	6	5	.....	.....			200	27. 14± . 14	2. 91± . 10	10. 73± . 36
P. graminis avenae....	*Avena sativa*.....	.....		.....		5	27	75	55	32	6	.....	.....			200	28. 50± . 15	3. 24± . 11	11. 34± . 38
P. graminis phleipratensis.	*Phleum pratense*....	.....		7		43	98	50	2	.....	.....	.....	.....			200	23. 95± . 12	2. 41± . 08	10. 01± . 34
P. graminis agrostis..	*Agrostis alba*.....	1		23		84	69	22	1	.....	.....	.....	.....			200	22. 37± . 12	2. 61± . 09	11. 67± . 39
		25μ		30μ		35μ	40μ	45μ	50μ	55μ	60μ	65μ	70μ					
Teliospores:																		
P. graminis tritici....	*Triticum vulgare*....	.....		.....		.....	12	16	28	20	17	6	1			100	51. 80±0. 49	7. 23±0. 34	13. 95±0. 67
P. graminis secalis....	*Agropyron repens*....	.....		.....		8	14	30	27	14	6	1	.....			100	47. 35± . 45	6. 65± . 32	14. 04± . 67
P. graminis avenae....	*Avena sativa*.....	.....		1		8	18	34	22	13	4	.....	.....			100	46. 15± . 43	6. 46± . 31	13. 85± . 66
P. graminis phleipratensis.	*Phleum pratense*....	.....		3		16	43	29	8	1	.....	.....	.....			100	41. 30± . 32	4. 78± . 23	11. 56± . 55
P. graminis agrostis..	*Agrostis alba*.....	1		7		24	34	23	9	2	.....	.....	.....			100	40. 30± . 40	5. 87± . 28	14. 56± . 70

TABLE IV.—*Variations and constants for width of spores of biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

Spores and biologic forms.	Host plants.	Spore classes according to width.													Total number.	Constants.		
		10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	Mean.	Standard deviation.	Coefficient of variability.
Aeciospores:																		
P. graminis tritici	*Berberis vulgaris*				2	4	14	21	7	2						50	15.66±0.10	1.07±0.07	6.83±0.46
P. graminis secalis	do		2	7	19	13	6	3								50	13.46± .11	1.17± .08	8.69± .58
P. graminis avenae	do			1	5	13	22	7	2							50	14.70± .10	1.02± .07	6.97± .47
P. graminis agrostis	do		3	12	21	11	3									50	12.98± .09	.97± .07	7.47± .50
Urediniospores:																		
P. graminis tritici	*Triticum vulgare*							2	6	20	44	83	26	15	4	200	19.79± .06	1.27± .04	6.42± .22
P. graminis secalis	*Secale cereale*						3	10	49	60	47	25	5	1		200	17.19± .06	1.26± .04	7.33± .25
P. graminis avenae	*Avena sativa*							3	7	17	38	70	42	17	6	200	19.94± .07	1.36± .05	6.90± .23
P. graminis phleipratensis	*Phleum pratense*				1	5	24	46	58	46	16	4				200	16.88± .06	1.32± .04	7.81± .27
P. graminis agrostis	*Agrostis alba*				4	27	52	72	35	10						200	15.68± .05	1.12± .04	7.16± .24
Teliospores:																		
P. graminis tritici	*Triticum vulgare*			1	3	6	14	20	27	16	6	6	1			100	16.67± .12	1.74± .08	10.42± .46
P. graminis secalis	*Agropyron repens*	1	3	8	10	20	25	18	8	4	3					100	14.77± .12	1.83± .09	12.37± .59
P. graminis avenae	*Avena sativa*			3	6	12	22	25	15	10	4	2	1			100	15.84± .12	1.78± .09	11.24± .54
P. graminis phleipratensis	*Phleum pratense*			2	5	13	25	29	17	7	2					100	15.63± .10	1.46± .07	9.35± .46
P. graminis agrostis	*Agrostis alba*	1	1	9	14	20	28	14	7	4	2					100	14.64± .12	1.72± .08	11.73± .56

AECIOSPORES

No previous record of aeciospore measurements of biologic forms of *P. graminis* could be found. The data available relate to the species as a whole, the assumption evidently having been that there was no difference in the size of aeciospores of the various biologic forms. Figures 1 and 2, however, show at a glance the variation existing between different biologic forms at this juncture in their life cycle. The variation is greater in figure 1, in which the curves for spore length are plotted. Here the class containing the greatest number of individuals in the *P. graminis tritici* curve is greater by 4 μ than the identical class in the agrostis curve,

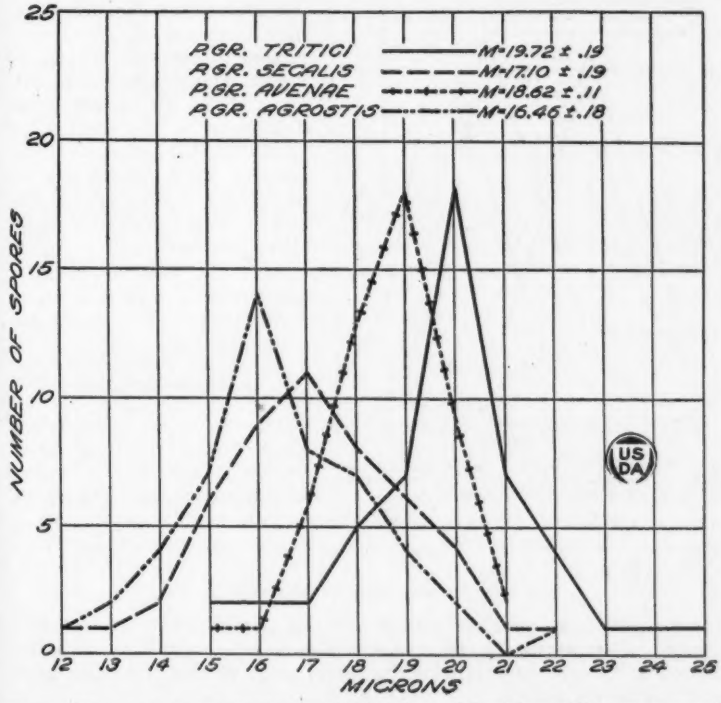


FIG. 1.—Differences in lengths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

greater by 3 μ than that in the secalis curve, and greater by only 1 μ than the homologous class in the avenae curve. Figure 2 presents a somewhat different picture. In this graph which represents curves for spore width, the greatest difference between any two classes containing the largest number of individuals is 3 μ, viz, *P. graminis tritici* and *P. graminis agrostis*. In *P. graminis secalis* and *P. graminis agrostis* the modes fall at exactly the same point, i. e., at 13 μ. An examination of the constants in the first section of Tables III and IV and of the differences in the means as presented in Table V will reveal not only the existence of a difference in the spore dimensions but in most cases also the distinct significance of such difference.

P. GRAMINIS TRITICI

The constants, together with their probable errors for the aeciospores of this biologic form, as given in the first section of Tables III and IV, respectively, show this form to have the largest arithmetical mean for both length and width, namely, $19.72 \pm 0.19 \times 15.66 \pm 0.10$ μ.

P. GRAMINIS SECALIS

The aeciospores of this biologic form differ considerably in both dimensions from those of the tritici form, the means being 17.10 ± 0.19 and 13.46 ± 0.11 μ, respectively. An examination of Table V will show that the difference in the means of these two biologic forms is, without doubt, significant for length as well as for width. The difference in the means of length divided by the probable error of this difference is 9.71, while the difference in the means of the width is 14.66 times greater than its probable error. The odds against the occurrence of such differences in random sampling are beyond comprehension.

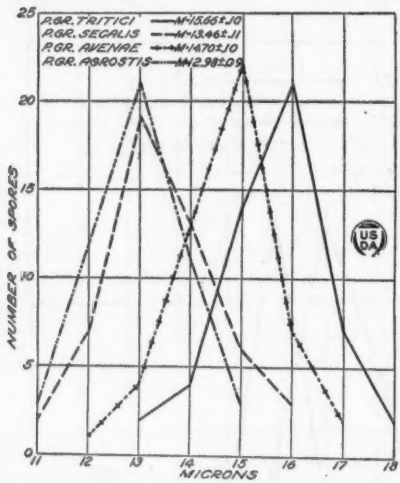


FIG. 2.—Differences in widths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

P. GRAMINIS AVENAE

This biologic form differs quite markedly from either of the above, although to a somewhat lesser extent. The differences, nevertheless, are very significant, as can readily be seen from Table V. The means of the aeciospores of this form are $18.62 \pm 0.11 \times 14.70 \pm 0.10$ μ.

P. GRAMINIS PHLEIPRATENSIS

Any attempt to produce aecial infection on different species and varieties of barberry with teliospores of this biologic form has invariably resulted in failure. A study of the aecial stage of this form therefore could not be made.

P. GRAMINIS AGROSTIS

As shown in Tables III and IV, this biologic form has aeciospores which are smaller in size than those of any of the other biologic forms. The means are $16.46 \pm 0.18 \times 12.98 \pm 0.09$ μ. The differences in the means between this form and tritici on the one hand, and avenae on the other hand, as indicated in Table V, are certainly significant. The difference between agrostis and secalis may or may not be due to random sampling, for the difference in the means of spore length is only 2.46 times greater than its probable error and the difference in width does not exceed 3.43 times the probable error. The odds against the normal occurrence of such differences are approximately 9 to 1 in the length differences, and about 45 to 1 in the width differences. It would be difficult therefore

to consider the differences between the agrostis and secalis forms as truly significant, although they might be considered as indicative. A greater number of measurements might possibly throw more light on this question.

The following table gives the differences in the means of the aeciospores of the four biologic forms discussed in the preceding paragraphs, as well as the ratios between these differences and their probable errors.

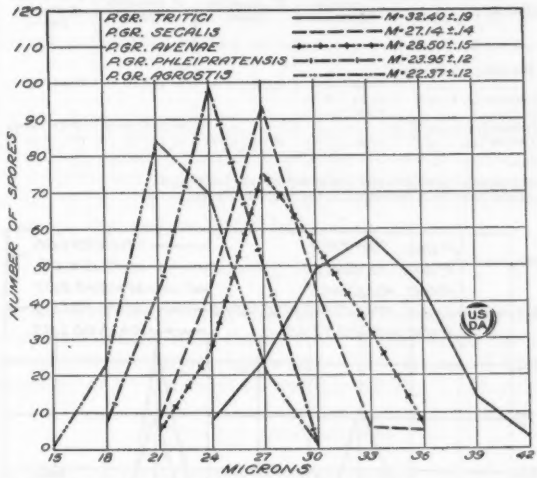


FIG. 3.—Differences in lengths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

TABLE V.—*Summary of differences in the means of aeciospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*.	2.62±0.27	2.20±0.15	9.71	14.66
P. graminis tritici and *P. graminis avenae*.	1.10± .22	.96± .14	5.00	6.86
P. graminis tritici and *P. graminis agrostis*.	3.26± .26	2.68± .13	12.53	20.61
P. graminis secalis and *P. graminis avenae*.	1.52± .22	1.24± .15	6.91	8.27
P. graminis secalis and *P. graminis agrostis*	.64± .26	.48± .14	2.46	3.43
P. graminis avenae and *P. graminis agrostis*	2.16± .21	1.72± .13	10.28	13.23

UREDINIOSPORES

There is considerably more information found in literature on the relative size of urediniospores of biologic forms of *P. graminis* than on either aeciospore or teliospore dimensions. An interesting phenomenon of spore size relationship becomes apparent when the curves plotted in figures 3 and 4 and the data compiled from previous investigations are examined.

It is that the tritici form possesses the longest urediniospores, the agrostis form the shortest, and the rest group themselves between these two extremes.

TABLE VI.—*Urediniospore dimensions of biologic forms of P. graminis as given by various authors*

Authority.	*P. graminis tritici.*	*P. graminis secalis.*	*P. graminis avenae.*	*P. graminis phleipratensis.*	*P. graminis agrostis.*
Eriksson and Henning (*3, p. 124 and 130*)	29–43×18–21	23–38×15–22	22–40×16–21	18–27×15–19	.....
Freeman and Johnson (*4, p. 27*)[1]	31.33×18.15	.....	.....	.....	.....
Stakman (*12, p. 27*)[1]	37.85×22.76	.....	.....	.....	.....
Stakman and Jensen (*13, p. 215*)[2]	.....	.....	19–35×16–24	17–31×15–23	.....
Stakman and Levine (*14, p. 40*)[2]	23–42×15–25	18–39×13–21	19–37×14–26	16–32×12–21	15–32×12–20

[1] These figures represent averages, size limits not having been given.
[2] Decimal fractions have been converted to the nearest integer.

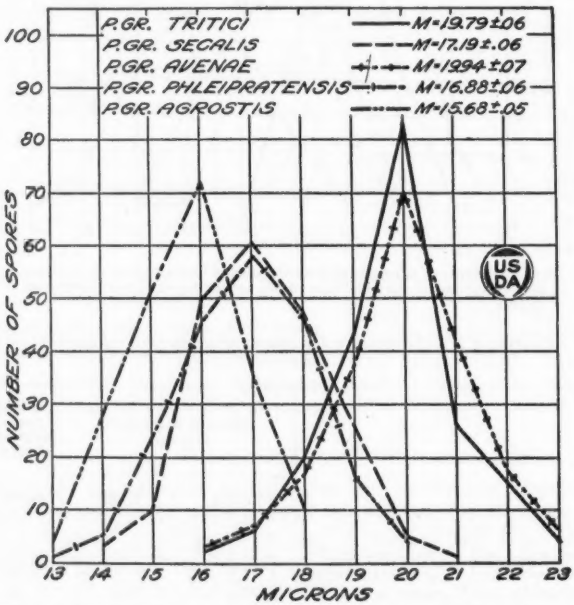


FIG. 4.—Differences in widths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

It will also be noted by examining the graphs and the measurements quoted that *P. graminis secalis* and *P. graminis avenae* differ but little in spore length, while the spore width of the latter is much the same as that of *P. graminis tritici.* It will be noted further that according to the graphs as well as the quotations, there is a similarity in the width of the urediniospores of the secalis and phleipratensis forms, but the two diverge noticeably in length. The constants, given in the second section of Tables III and IV, and the differences in the means, presented in

Table VII, bring out not only the variations of the urediniospore dimensions but also the significance of these variations whenever and wherever they are found.

P. GRAMINIS TRITICI

That this biologic form has the longest urediniospores is confirmed by the constants, together with their probable errors, given in the second section of Tables III and IV. In width, the urediniospores are practically the same as those of the oat-rust form. The means for *P. graminis tritici* fall at $32.40 \pm 0.19 \times 19.79 \pm 0.06\mu$.

P. GRAMINIS SECALIS

The rye-rust urediniospores are both shorter and narrower than those of the wheat rust as shown by the means, $27.14 \pm 0.14 \times 17.19 \pm 0.06\mu$, there being a difference of over 5μ in length and more than 3μ in width. There can be no doubt whatever concerning the significance of this difference, as pointed out in Table VII.

P. GRAMINIS AVENAE

Although the urediniospores of this biologic form are only a little more than 1 micron (μ) longer than those of the secalis form, the difference, nevertheless, seems to be quite significant. The means of the urediniospore dimensions of *P. graminis avenae* are $28.50 \pm 0.15 \times 19.94 \pm 0.07\mu$.

P. GRAMINIS PHLEIPRATENSIS

The means of this biologic form are $23.95 \pm 0.12 \times 16.88 \pm 0.06\mu$. The differences between the means for both width and length of this biologic form and those of *P. graminis tritici* and *P. graminis avenae* are quite significant. The difference in the means, of length only, of the timothy rust and the rye rust is also noteworthy. (Table VII).

P. GRAMINIS AGROSTIS

The urediniospores of this biologic form are without any doubt smaller than those of any of the other forms. This is demonstrated by the curves plotted in figures 3 and 4 and confirmed by the constants in Tables III and IV, and also by the differences in the means as shown in Table VII. The means of the redtop-rust urediniospores are $22.37 \pm 0.12 \times 15.68 \pm 0.05\mu$.

The differences in the means of the urediniospores of the biologic forms of *P. graminis* and the ratios between these differences and their probable errors are summarized in Table VII.

TABLE VII.—*Summary of differences in the means of urediniospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*	5.26±0.24	2.60±0.08	21.92	32.50
P. graminis tritici and *P. graminis avenae*	3.90± .24	.15± .09	16.25	1.67
P. graminis tritici and *P. graminis phleipratensis*	8.45± .22	2.91± .08	38.38	36.38
P. graminis tritici and *P. graminis agrostis*	10.03± .22	4.11± .08	46.82	51.38
P. graminis secalis and *P. graminis avenae*	1.36± .21	2.75± .09	6.48	30.56
P. graminis secalis and *P. graminis phleipratensis*	3.19± .18	.31± .08	17.72	3.88
P. graminis secalis and *P. graminis agrostis*	4.77± .18	1.51± .08	26.50	18.88
P. graminis avenae and *P. graminis phleipratensis*	4.55± .19	3.06± .09	23.93	34.00
P. graminis avenae and *P. graminis agrostis*	6.13± .19	4.26± .09	32.25	47.34
P. graminis phleipratensis and *P. graminis agrostis*	1.58± .17	1.20± .08	9.39	15.00

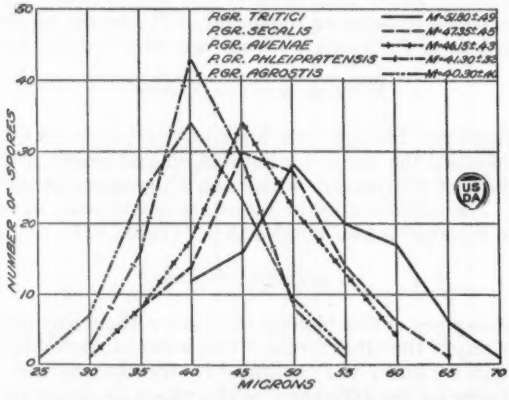


FIG. 5.—Differences in lengths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

TELIOSPORES

Published data on teliospore dimensions can be found concerning only one biologic form of stem rust and that is timothy rust. The evident reason is because this form had been considered a distinct species. The other data available refer to the species *P. graminis* as a unit. The various authors consulted agree on the range in the size of teliospores of timothy rust. This is given as 38–52 × 14–16μ. Most authors, however, disagree on the size limits of the teliospores of *P. graminis*, perhaps on account of a difference in the biologic forms which had been studied. The curves plotted in figures 5 and 6 point to the existence of a difference in size of the teliospores of the various biologic forms. In general there seems to be a fairly distinct parallellism in the size of the teliospores of the various

forms of *P. graminis* and the size of the urediniospores and aeciospores of the same forms. As in the case of the last two spore types, so in the teliospores, *P. graminis tritici* and *P. graminis agrostis* constitute the extremes with respect to spore size. Figures 5 and 6 show very clearly that the teliospores of the wheat rust are larger in every way than those of any of the other forms. The secalis and avenae forms are practically the same in length but differ noticeably in width. On the other hand, the rusts of rye and of redtop vary insignificantly in spore width. In length the agrostis form approaches that of phleipratensis, but is appreciably different from it in width. The constants given in the third section of Tables III and IV and the differences in the means presented in Table VIII show when these similarities and differences are real and significant and when they are only apparent and inconclusive.

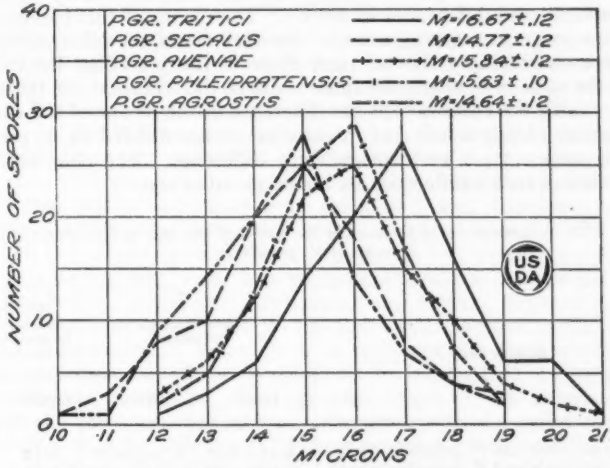


FIG. 6.—Differences in widths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

P. GRAMINIS TRITICI

There is a difference of at least 5 microns between the mode for length of the teliospores of this biologic form and the mode for the longitudinal measurement of any other biologic form. A similar difference is found in the means for length. The greatest difference in the width of the teliospore between this and any other form is about one micron for either mode or means. The means for this biologic form, as given in section 3 of Tables III and IV, are $51.80 \pm 0.49 \times 16.67 \pm 0.12\mu$.

P. GRAMINIS SECALIS

The difference between this biologic form and the one described above can readily be seen by comparing their means, which for the secalis form are $47.35 \pm 0.45 \times 14.77 \pm 0.12\mu$. The difference in the means of the rye-rust and wheat-rust forms is 6.64 times its probable error for length and 11.23 times greater than its probable error for width. Consequently, the difference must be considered as truly significant.

P. GRAMINIS AVENAE

The means for this form were determined as $46.15 \pm 0.43 \times 15.84 \pm 0.12\ \mu$. The difference in means between this biologic form and *P. graminis tritici* is obviously quite significant; not so in the case of the difference in the means of the avenae and the secalis forms, however. Here the difference in the means of the length of the biologic forms is only 1.94 times greater than its probable error and therefore insignificant, because such a difference is likely to occur naturally about once in every five random samples. There is, however, a significant difference in the means of the width of these two forms, it being 6.30 times greater than its probable error.

P. GRAMINIS PHLEIPRATENSIS

A reverse condition prevails in the timothy rust, as seen by examining its constants and differences in means. The means of this biologic form fall at $41.30 \pm 0.32 \times 15.63 \pm 0.10\ \mu$. In other words, the teliospores of this form are considerably shorter than those of the preceding three forms, but of the same or of nearly the same width as the teliospores of the *avenae* form. Table VIII shows that the difference in the means of the width of *P. graminis phleipratensis* and *P. graminis avenae* divided by its probable error is only 1.31, a very insignificant difference. The chances for an occurrence of such a difference are about 38 out of 100.

TABLE VIII.—*Summary of differences in the means of the sizes of teliospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*	4.45±0.67	1.90±0.17	6.64	11.23
P. graminis tritici and *P. graminis avenae*	5.65± .65	.83± .17	8.69	4.88
P. graminis tritici and *P. graminis phleipratensis*	10.50± .59	1.04± .16	17.80	6.50
P. graminis tritici and *P. graminis agrostis*	11.50± .63	2.03± .17	18.25	11.93
P. graminis secalis and *P. graminis avenae*	1.20± .62	1.07± .17	1.94	6.30
P. graminis secalis and *P. graminis phleipratensis*	6.05± .55	.86± .16	11.00	5.37
P. graminis secalis and *P. graminis agrostis*	7.05± .60	.13± .17	11.74	.77
P. graminis avenae and *P. graminis phleipratensis*	4.85± .54	.21± .16	8.98	1.31
P. graminis avenae and *P. graminis agrostis*	5.85± .59	1.20± .17	9.92	7.06
P. graminis phleipratensis and *P. graminis agrostis*	1.00± .51	.99± .16	1.96	6.18

P. GRAMINIS AGROSTIS

The redtop rust has the smallest teliospores of any of the biologic forms, although the difference is not direct or absolute. These spores are much shorter than those of either the wheat, rye, or oat rust, but only a little shorter than those of the timothy rust. On the other hand, they are appreciably narrower than those of the wheat, oat, and timothy forms, but much the same in width as the teliospores of the rye rust.

This characteristic variation is brought out more clearly by an analysis of the differences in the means than by a mere consideration of the constants. The means of this biologic form are $40.30 \pm 0.40 \times 14.64 \pm 0.12 \mu$. The difference in the means between the agrostis and the tritici forms is significant for both dimensions. Between agrostis and secalis, however, the difference in the means is significant for length only. Again, between the agrostis and avenae forms, the difference is significant for both length and width. The difference in the length of teliospore means of the agrostis and phleipratensis forms has no value as it is only 1.96 times greater than its probable error. The difference in the width, however, appears to be very significant because it exceeds its probable error by 6.18 times.

EFFECT OF ENVIRONMENTAL CONDITIONS ON THE MORPHOLOGY OF RUST SPORES

It has been reported (*14*) that resistant host plants and other unfavorable environmental conditions affecting the normal development and vigor of the rust fungus tend to change appreciably the size of its uredinio-spores. However, when normal conditions are reestablished, the uredinio-spores of the following generation are of the original dimensions. This experiment was elaborated and extended to the aeciospores and teliospores. Curves for the distribution of the spores studied were plotted into classes; constants were calculated; and differences in the means of varying conditions established. Tables IX and X represent the spore-size variations for length and width, respectively, as well as the constants together with their probable errors, for the several spore types as affected by different host plants and varying physical factors. The classes in these tables differ in the same order as those in Tables III and IV. The curves in figures 7 to 12 represent the distribution into their respective classes of the spores of the various biologic forms, grown under different conditions. The probable errors of the differences in the means of the spore measurements, together with the ratios obtained from a division of the differences by their probable errors, and the differences in the means themselves are all given in Tables XI and XII.

HOST PLANTS

Species and varieties which are congenial to a certain biologic form of *P. graminis* can not, under favorable cultural conditions, exert any perceptible influence on the spore morphology of that biologic form. However, even under identically the same environmental conditions, the spores of any biologic form will be reduced in size when cultured on resistant varieties. This is substantiated by measurements made of spores grown simultaneously on susceptible and resistant hosts.

SUSCEPTIBLE HOSTS

Aeciospores of *P. graminis secalis* were produced on *Berberis vulgaris* and *B. sieboldii* Miq., two equally susceptible species. The inoculations were made with teliospores of the same origin. The difference in the size of the two sets of aeciospores was practically negligible and highly insignificant (fig. 7 and 8). The means of the spores on *B. vulgaris* were $17.10 \pm 0.19 \times 13.46 \pm 0.11$ μ, as compared with $17.44 \pm 0.15 \times 13.30 \pm 0.09$ μ for the means on *B. sieboldii*.

TABLE IX.—*Variations and constants for length of spores of biologic forms of Puccinia graminis grown on different host plants and under varying environmental conditions*

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to length.												Total number.	Constants.		
			11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ		Mean.	Standard deviation.	Coefficient of variability.
	Aeciospores:																	
1	*P. graminis secalis*	*Berberis vulgaris*	...	1	1	2	6	9	11	8	6	4	1	1	50	17. 10±0. 19	2. 00±0. 13	11. 70±0. 79
2	Do	*Berberis sieboldii*	...	...	...	1	5	7	15	9	7	5	1	...	50	17. 44± . 15	1. 58± . 11	9. 25± . 61
3	*P. graminis agrostis*	*Berberis vulgaris*	...	1	2	4	7	14	8	7	4	2	0	1	50	16. 46± . 18	1. 93± . 13	11. 73± . 79
4	Do	*Berberis brevipaniculata.*	2	8	11	15	9	5	...	...	...	...	...	...	50	13. 72± . 12	1. 31± . 09	9. 56± . 65

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to length.										Total number.	Constants.		
			15μ	18μ	21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ		Mean.	Standard deviation.	Coefficient of variability.
	Urediniospores:															
5	*P. graminis avenae*	*Avena sativa*	...	...	5	27	75	55	32	6	...	...	200	28. 50± . 15	3. 24± . 11	11. 34± . 38
6	Do	*Bromus tectorum*	...	1	32	43	23	1	...	...	...	...	100	23. 73± . 16	2. 37± . 11	9. 97± . 48
7	*P. graminis phleipratensis.*	*Phleum pratense*	...	7	43	98	50	2	...	...	...	...	200	23. 95± . 12	2. 41± . 08	10. 01± . 34
8	Do	*Dactylis glomerata*	...	1	27	46	26	...	...	...	...	...	100	23. 91± . 15	2. 26± . 11	9. 48± . 46
9	*P. graminis tritici*	Normal conditions	...	...	...	8	24	49	58	44	14	3	200	32. 40± . 19	3. 89± . 13	12. 00± . 41
10	Do	Excessive heat	...	...	...	10	29	40	18	3	...	...	100	29. 25± . 20	2. 89± . 14	9. 88± . 68
11	Do	Deficient light	...	...	1	13	30	37	15	4	...	...	100	28. 92± . 21	3. 13± . 15	10. 76± . 51
12	Do	Drought	...	...	...	8	19	37	21	14	1	...	100	30. 51± . 23	3. 47± . 17	11. 38± . 54

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to length.								Total number.	Constants.		
			35μ	40μ	45μ	50μ	55μ	60μ	65μ	70μ		Mean.	Standard deviation.	Coefficient of variability.
	Teliospores:													
13	*P. graminis tritici*	*Triticum vulgare*	.....	12	16	28	20	17	6	1	100	51. 80± . 49	7. 23± . 34	13. 95± . 67
14	Do	*Triticum dicoccum*	3	18	42	27	10	.....	.....	.....	100	46. 15± . 32	4. 79± . 23	10. 38± . 49

TABLE X.—*Variations and constants for width of spores of biologic forms of Puccinia graminis grown on different host plants and under varying environmental conditions*

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to width.															Total number.	Constants.		
			10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ		Mean.	Standard deviation.	Coefficient of variability.
	Aeciospores:																				
1	*P. graminis secalis.*	*Berberis vulgaris*	...	2	7	19	13	6	3	...	...	...	...	...	...	...	...	50	13.46±0.11	1.17±0.08	8.69±0.58
2	Do	*Berberis sieboldii*	...	1	7	23	14	5	...	...	...	...	...	...	...	...	...	50	13.30± .09	.90± .06	6.77± .46
3	*P. graminis agrostis.*	*Berberis vulgaris*	...	3	12	21	11	3	...	...	...	...	...	...	...	...	...	50	12.98± .09	.97± .07	7.47± .50
4	Do	*Berberis brevipaniculata.*	2	11	30	6	1	...	...	...	...	...	...	...	...	...	...	50	11.86± .07	.75± .05	6.26± .42
	Urediniospores:																				
5	*P. graminis avenae.*	*Avena sativa.*	...	...	...	...	...	...	3	7	17	38	70	42	17	6	...	200	19.94± .07	1.36± .05	6.90± .23
6	Do	*Bromus tectorum.*	...	...	...	...	...	...	1	10	22	34	29	4	...	...	...	100	18.92± .07	1.08± .05	5.69± .37
7	*P. graminis phleipratensis.*	*Phleum pratense*	...	...	...	1	5	24	46	58	46	16	4	...	...	...	...	200	16.88± .06	1.32± .04	7.81± .27
8	Do	*Dactylis glomerata.*	...	...	...	...	2	8	22	33	22	11	2	...	...	...	...	100	17.06± .08	1.25± .06	7.32± .35
9	*P. graminis tritici.*	Normal conditions.	...	...	...	...	...	...	2	6	20	44	83	26	15	4	...	200	19.79± .06	1.27± .04	6.42± .22
10	Do	Excessive heat	...	...	...	...	...	...	1	6	14	18	35	17	6	2	1	100	19.71± .10	1.45± .07	.7± .35
11	Do	Deficient light	...	...	...	...	...	...	1	11	20	44	15	8	1	...	...	100	19.89± .08	1.13± .05	5.69± .27
12	Do	Drought	...	...	...	...	...	...	4	18	36	28	13	1	...	...	...	100	19.31± .07	1.06± .05	5.51± .26
	Teliospores:																				
13	*P. graminis tritici.*	*Triticum vulgare*	...	...	1	3	6	14	20	27	16	6	6	1	...	...	...	100	16.67± .12	1.74± .08	10.42±.46
14	Do	*Triticum dicoccum.*	...	...	1	3	6	16	21	28	13	6	4	2	...	...	...	100	16.57± .12	1.73± .08	10.43±.50

Similar results were obtained with urediniospores of *P. graminis phleipratensis* of identical origin grown simultaneously on timothy, *Phleum pratense*, and orchard grass, *Dactylis glomerata* L. As can readily be seen from experiments 7 and 8 of Tables IX and X, the spores were alike in length and width; the means for the urediniospores developed on the timothy were $23.95 \pm 0.12 \times 16.88 \pm 0.06$ μ, while the means for the urediniospores obtained on the orchard grass were $23.91 \pm 0.15 \times 17.06 \pm 0.08$ μ. Thus the means in both cases differed only slightly.

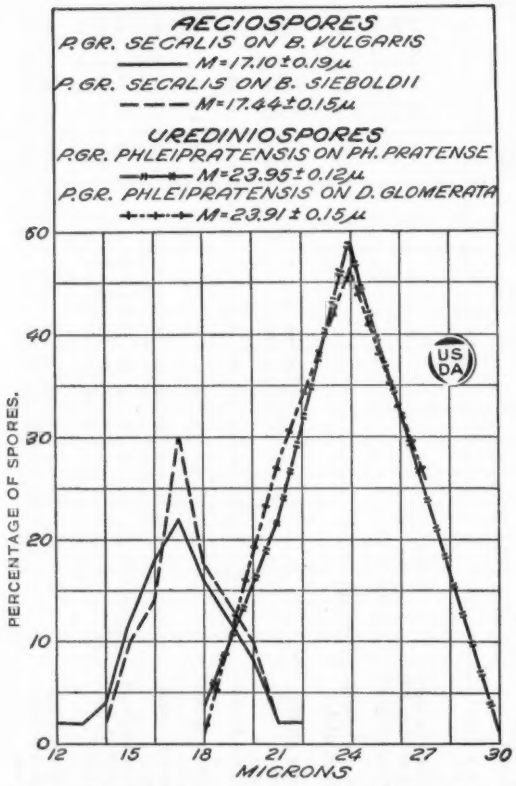


Fig. 7.—Uniformity in length of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

RESISTANT HOSTS

As a rule the width of spores is more or less constant under all circumstances. However, a reduction in both length and width as a result of the effect of resistant hosts was obtained in aeciospores of *P. graminis agrostis* produced on *Berberis brevipaniculata* Schneid. and in urediniospores of *P. graminis avenae* grown on *Bromus tectorum*. A reduction in length only occurred in the teliospores of *P. graminis tritici* found on emmer, *Triticum dicoccum* Schr. Figures 9 and 10 illustrate these variations very distinctly.

The aeciospores of the agrostis form on *B. vulgaris* possessed the following means: $16.46 \pm 0.18 \times 12.98 \pm 0.09$ μ, while the means of these aeciospores on *B. brevipaniculata* were $13.72 \pm 0.12 \times 11.86 \pm 0.07$ μ. The difference between the two conditions, as shown in Table XI, is 12.45 times greater than the probable error for length and 10.18 times greater than that for width, which without any doubt is a very significant difference.

Bromus tectorum L. produced a similar effect on the urediniospores of *P. graminis avenae*. The means of this biologic form on *Avena sativa*

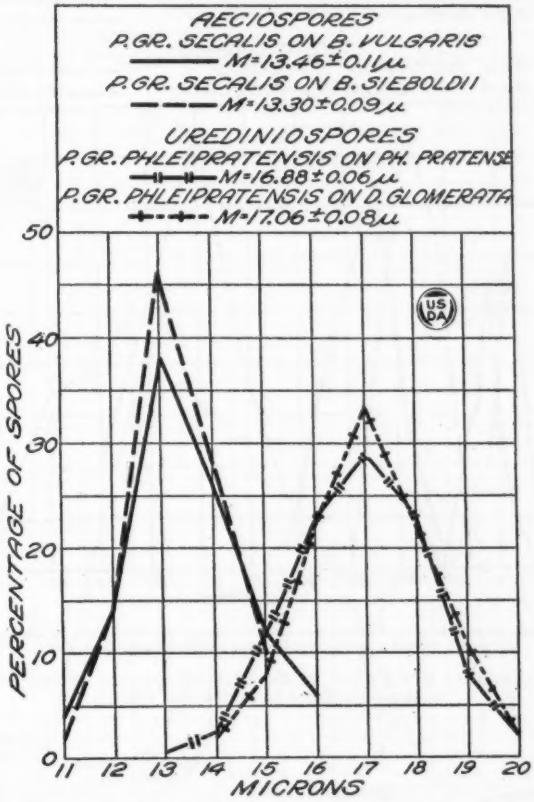


Fig. 8.—Uniformity in widths of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

were $28.50 \pm 0.15 \times 19.94 \pm 0.07$ μ, while on *B. tectorum* they were $23.73 \pm 0.16 \times 18.92 \pm 0.07$ μ. The difference in the means of the urediniospores in the two cases was 21.67 times its probable error for length and 10.20 times for width; both are differences as significant as can be desired.

The teliospores on the common wheat were over 5 μ longer than those on emmer, but practically the same in width. The means for the former were $51.80 \pm 0.49 \times 16.67 \pm 0.12$ μ, while for the latter they were $46.15 \pm 0.32 \times 16.57 \pm 0.12$ μ. The difference in the means of the length only was significant.

Table XI shows the differences in the means of spores of biologic forms of stem rust grown on susceptible and resistant hosts, also the ratios between these differences and their probable errors.

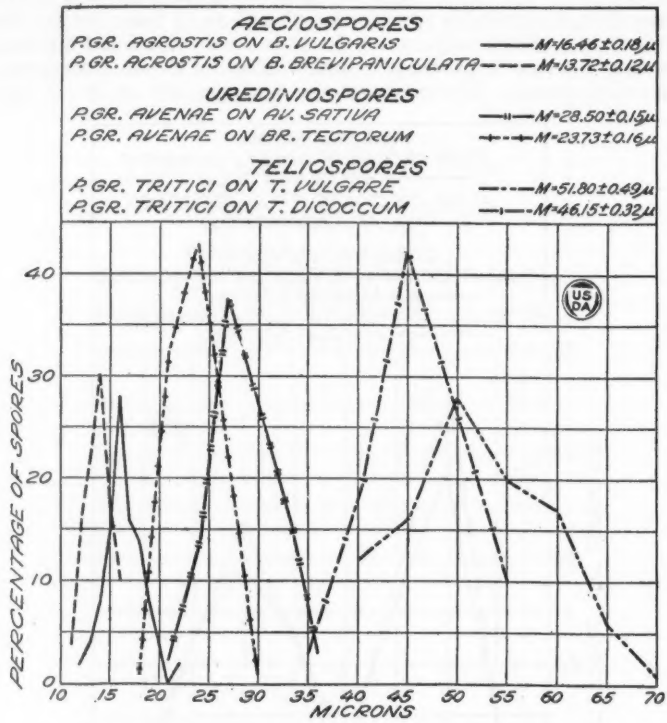

AECIOSPORES
P. GR. AGROSTIS ON B. VULGARIS —— M=16.46±0.18μ
P. GR. AGROSTIS ON B. BREVIPANICULATA — — M=13.72±0.12μ
UREDINIOSPORES
P. GR. AVENAE ON AV. SATIVA —+— M=28.50±0.15μ
P. GR. AVENAE ON BR. TECTORUM —+—+— M=23.73±0.16μ
TELIOSPORES
P. GR. TRITICI ON T. VULGARE —·— M=51.80±0.49μ
P. GR. TRITICI ON T. DICOCCUM —·—·— M=46.15±0.32μ
PERCENTAGE OF SPORES
0
10
20
30
40
10 15 20 25 30 35 40 45 50 55 60 65 70
MICRONS
USDA

Fig. 9.—Differences in lengths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and on resistant host plants.

TABLE XI.—*Summary of differences in the means of spores of biologic forms of P. graminis as affected by various host plants*

Biologic forms.	Host plants.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
		Length.	Width.	Length.	Width.
P. graminis secalis (aeciospores)	*Berberis vulgaris* and *Berberis sieboldii*	0.34±0.24	0.16±0.14	1.42	1.14
P. graminis phleipratensis (urediniospores)	*Phleum pratense* and *Dactylis glomerata*	.04± .19	.18± .10	.21	1.80
P. graminis agrostis (aeciospores)	*B. vulgaris* and *B. brevipaniculata*	2.74± .22	1.12± .11	12.45	10.18
P. graminis avenae (urediniospores)	*Avena sativa* and *Bromus tectorum*	4.77± .22	1.02± .10	21.67	10.20
P. graminis tritici (teliospores)	*Triticum vulgare* and *T. dicoccum*	5.65± .59	.10± .17	9.75	.59

PHYSICAL FACTORS

Unfavorable cultural conditions affect the virulence and spore size of the rust fungus to an appreciable extent, especially the quantity of rust produced and the length of the spores. The inherent nature of infection and the width of the spores are rarely and only slightly changed, if at all. For obvious reasons the effect of environment on the uredinial stage

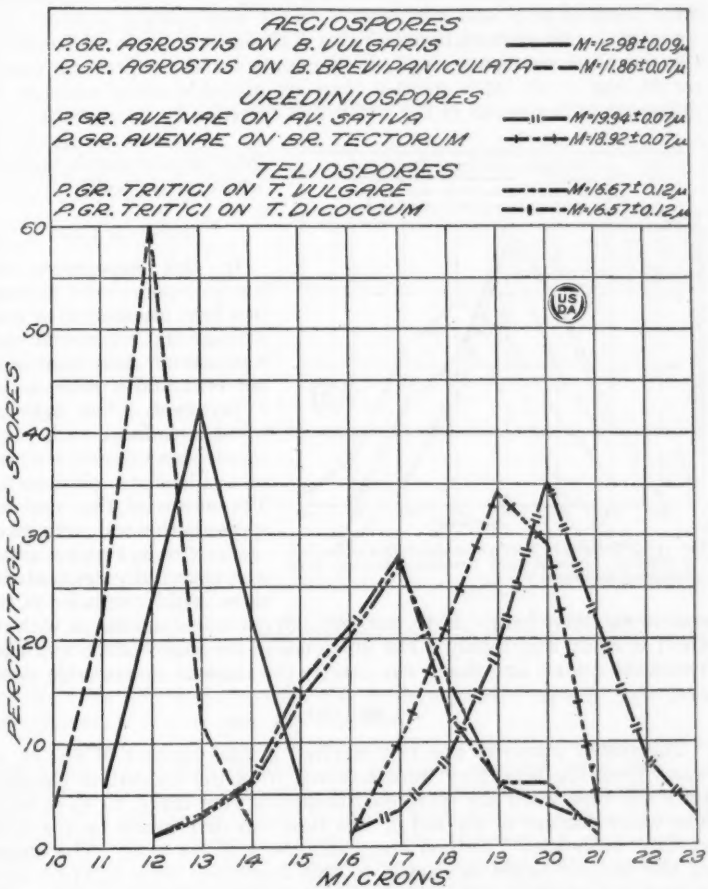

AECIOSPORES
P.GR. AGROSTIS ON B. VULGARIS M=12.98±0.09μ
P.GR. AGROSTIS ON B. BREVIPANICULATA M=11.86±0.07μ
UREDINIOSPORES
P.GR. AVENAE ON AV. SATIVA M=19.94±0.07μ
P.GR. AVENAE ON BR. TECTORUM M=18.92±0.07μ
TELIOSPORES
P.GR. TRITICI ON T. VULGARE M=16.67±0.12μ
P.GR. TRITICI ON T. DICOCCUM M=16.57±0.12μ
PERCENTAGE OF SPORES
0 10 20 30 40 50 60
10 11 12 13 14 15 16 17 18 19 20 21 22 23
MICRONS
USDA

Fig. 10.—Differences in widths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and resistant host plants.

alone was studied. Excessive heat more effectively and more conclusively caused the urediniospores of the wheat rust to become shorter than did very low temperature. Deficiency in sunlight and in soil moisture appeared to be equally influential in diminishing the size of the urediniospores. In all of these cases as well as in the control experiment

an extremely susceptible variety of wheat, Haynes Bluestem (Minnesota No. 169), was used as the host plant. Figures 11 and 12 contain curves showing the variations produced by the various conditions.

EXCESSIVE HEAT

The means of the spores in the control experiment were $32.40 \pm 0.19 \times 19.79 \pm 0.06$ μ. The means of the urediniospores cultured in high temperature (average for the generation being 81.8° F. with a maximum daily mean of 92.3° and minimum of 76.4°) were $29.25 \pm 0.20 \times 19.71 \pm 0.10$ μ. An examination of Table XII shows that the difference in the means of the spore length in the control and temperature experiments was 11.25 times greater than its probable error, whereas the difference in the means of the width was exceeded by its probable error by 1.5 times; a significant difference for length, but none whatever for width.

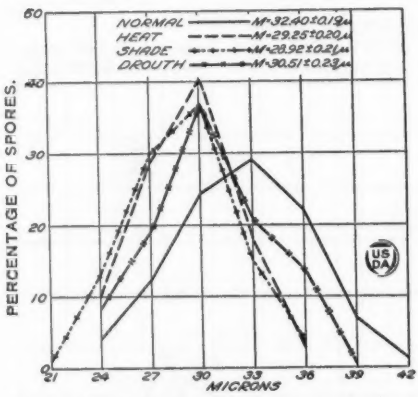


FIG. 11.—Differences in lengths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

DEFICIENT LIGHT

In this experiment the urediniospores were cultured in a light intensity of a total average of 3.1 per cent with a maximum daily limit of 10 per cent and a minimum of 2 per cent. The light intensity readings were made three times a day with the aid of a Clements photometer. The means of the urediniospores in this test were $28.92 \pm 0.21 \times 19.89 \pm 0.08$ μ, which were practically identical with those which prevailed in the case of excessive heat. Here, too, the difference was significant with respect to spore length only. The difference in the means of the width between the control and shade was exactly the same as its probable error.

DROUGHT

The rusted plants in this test received a total amount of 100 cc. of water from the time they were removed from the incubation chamber until the time when the spore measurements were made, 14 days later. The water content of the soil at this time was determined on the basis of the "oven-dried" method and measured 5.38 per cent. The means of the urediniospores subjected to this condition were $30.51 \pm 0.23 \times 19.31 \pm 0.07$ μ. Comparing these means with those of the control spores, we find a difference in both length and width which appears to be significant.

A summary of the differences and their relations to the probable errors of the differences is given in Table XII.

TABLE XII.—*Summary of differences in the means of urediniospores of P. graminis tritici subjected to various environmental conditions*

Environmental conditions favorable except for—	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	*Width.*	*Length.*	*Width.*
Excessive heat (average 81.8° F.).....	3.15±0.28	0.08±0.12	11.25	0.67
Deficient light (average 3.1 per cent)....	3.48± .28	.10± .10	12.42	1.00
Drought (water content 5.38 per cent)....	1.89± .30	.48± .09	6.30	5.34

GENERAL DISCUSSION

No definite statistical information concerning the dimensions of the different types of spores of the biologic forms of stem rust existed prior to this study. Measurements of aeciospores, urediniospores, and teliospores, developed under different sets of conditions and on different host plants, were made with the aim of supplying such information. Biometric methods were employed with a view of ascertaining the significance of the variations discovered. These methods were used merely as a means to ascertain more about the nature and behavior and the identity of the different biologic forms. That results thus obtained are more reliable than a mere mass of individual measurements is now generally conceded.

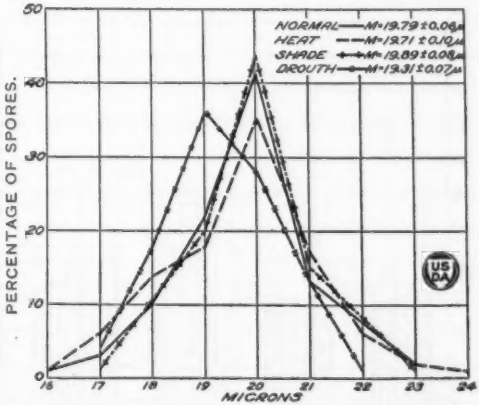


FIG. 12.—Differences in widths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

The statistical data presented in this paper, in addition to corroborating the empirical results obtained by previous workers concerning the differences in the size of urediniospores, also indicate very strongly the existence of similar differences in the aeciospores and teliospores of the biologic forms of *Puccinia graminis*. The differences in magnitude of the various types of spores seem to be quite marked, although they do not occur in any definite order or logical sequence. Sometimes a significant difference appears in both length and width of spores; on other occasions the difference can be detected in the spore length only, the width being almost identically the same, and vice versa.

A numerical difference alone may or may not be significant in itself. A variation of 1 micron in the means of two measurements, for instance, may in one case be ascribed to experimental error and on another occasion constitute a fundamental difference. The difference in the means of teliospores of *P. graminis phleipratensis* and *P. graminis agrostis* is a

case which can well illustrate this point. The two biologic forms differ by exactly 1.00 ± 0.51 μ in the means of length and by 0.99 ± 0.16 μ in the means of width. The difference in length was probably due to random sampling, as it was only 1.96 times greater than its probable error, whereas the difference in width appeared to be rather significant, because it exceeded its probable error by 6.18 times.

Two numerical differences of unequal magnitude, on the other hand, may sometimes be of identical or nearly identical significance. Thus, for instance, the urediniospores of *P. graminis tritici* exceed those of *P. graminis phleipratensis* by 8.45 ± 0.22 μ in the means of length, but the difference in the means of width is only 2.91 ± 0.08 μ. The difference in the means of length divided by its probable error is 38.38, and that of the width is 36.38. In general, the greater the numerical difference the greater its significance, although in certain cases the situation may be reversed. An excellent illustration of this phenomenon is furnished by the difference in the means of urediniospores of *P. graminis tritici* and *P.*

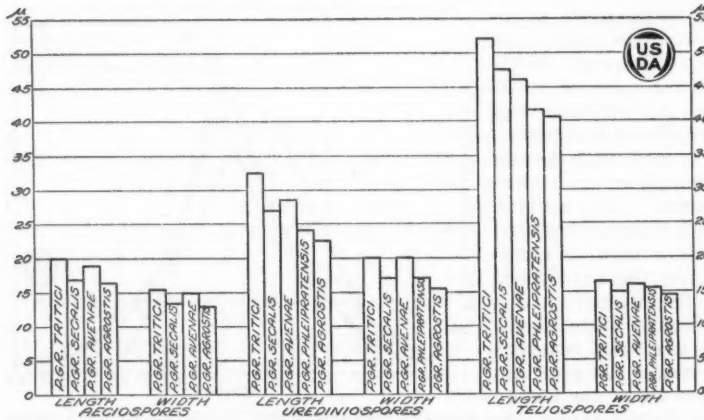


FIG. 13.—Comparisons between the means of lengths and widths of aeciospores, urediniospores, and teliospores, respectively, of biologic forms of *Puccinia graminis*.

graminis secalis. The means of the wheat-rust spores were 5.27 ± 0.24 μ longer and 2.60 ± 0.08 μ wider than those of the urediniospores of the rye rust. But the difference in the means of length exceeded its probable error 21.92 times as compared with 32.50 times for the excess in the case of the difference in width. Thus the important factor in determining the value and significance of differences is not so much the numerical deviation as the probable error of these differences.

The comparative differences in spore dimensions of the various biologic forms are illustrated by photomicrographs and schematic drawings in Plates I and II. Figure 13 represents diagrammatically the apparent correlation between the means of the different forms, while figure 14 shows the rather consistent parallelism in the means of the various spore types.

Although the size of spores is specific for each biologic form, the spores are not rigidly fixed either in size or in shape. Resistant host plants and other adverse environmental conditions, affecting the normal development and vigor of the rust fungus, also affect the size and shape of the

spores. Plate II, F, G, shows the response of the aecial infection of the redtop rust to two species of Berberis, one susceptible, *B. vulgaris* (F), and one resistant, *B. brevipaniculata* (G). The effect of these hosts on the size of the aeciospores is graphically illustrated in figures 9 and 10. Urediniospores and teliospores were also affected in a similar manner. This effect, however, is not permanent; it lasts only as long as the unfavorable circumstances prevail. This is especially true in the case of urediniospores. As soon as the rust is transferred to a congenial host plant and favorable cultural conditions are reestablished it again develops normally and the spores attain their normal dimensions.

The ill effect of excessive heat, insufficient light, and deficient soil moisture was quite definitely proved for urediniospores. This would lead to the assumption that aeciospores and teliospores are probably likewise affected by unfavorable cultural conditions.

Host plants belonging to different species and varieties are not in themselves sufficient to cause a change in the normal development of the rust fungus or in the size and shape of the spores unless they vary mark-

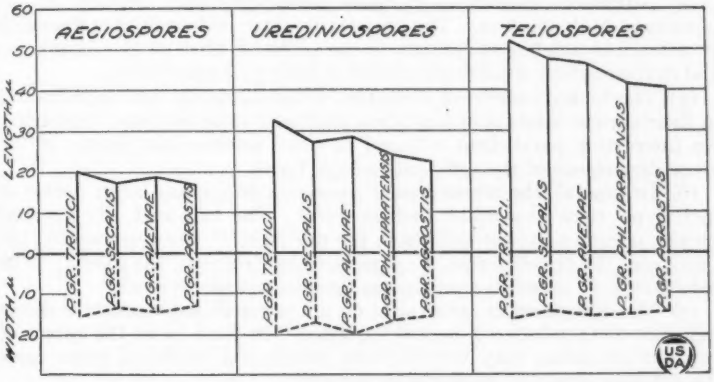


FIG. 14.—Parallelism between the means for lengths and widths of the different spores of biologic forms of *Puccinia graminis*.

edly in the degree of susceptibility to a biologic form. Plate II, D, E, shows an equal amount of aecial infection of *P. graminis secalis* on *B. vulgaris* and *B. sieboldii*. The uniformity in spore size of both cases is illustrated by graphs in figures 7 and 8.

Besides differences in spore size, deviations in the shape of the spores of the various biologic forms were also observed. These were described by Stakman and Levine (*14, p. 48*) as especially pronounced in the urediniospores. Somewhat less pronounced differences in shape were found by the writer in the aeciospores and teliospores, particularly the latter. Unfortunately the necessary statistical data were not available for calculating the ratios of length to width in the different types of spores, and hence constants could not be computed, nor could differences in mean of diameter be established. This is all the more regrettable because of the full realization of the importance of expressing quantitatively the differences occurring not only in the size but also in the shape of spores. A comprehensive method for this purpose has been described by Rosenbaum (*10, p. 250*).

SUMMARY

(1) In studying the nature and behavior of biologic forms careful uniform technic and pure rust strains have been used in order to reduce to a minimum the possibility of experimental error.

(2) Spore measurements can be employed as an additional aid in identifying the biologic forms of *P. graminis*, provided a sufficiently large number of spores are measured for both length and width, and the spores studied are developed on congenial hosts and under favorable conditions. The different measurements should be grouped in classes for length and width, respectively.

(3) On account of the variability in size of the different kinds of spores of each biologic form, a more ready comparison can be made by the use of biometric constants than by a mere mass of individual measurements. By the use of these constants the identity and relationship as well as the nature and behavior of a given biologic form may be further confirmed. Such biometric constants have been calculated and are incorporated in the respective tables.

(4) Numerical differences in spore dimensions may or may not be significant in themselves. The important factor in determining the value of numerical differences consists in the probable error of the differences and in the relation of these differences to their probable errors.

(5) The biologic forms of stem rust differ markedly and significantly in their various kinds of spores when produced under uniform conditions. An interesting parallelism is found to exist between the means of the spore dimensions of the different biologic forms.

(6) In general, the wheat rust, *P. graminis tritici*, has larger spores of each type than any other biologic form. The oat rust, *P. graminis avenae*, occupies the second place; the rye rust, *P. graminis secalis*, the third; and the timothy rust, *P. graminis phleipratensis*, the fourth. The redtop rust, *P. graminis agrostis*, has the smallest spores of all.

(7) The differences in spore sizes do not occur in any consistent direction, nor do they follow a logical sequence. In some cases the spores of two biologic forms may vary in both length and width, in other cases the differences may be in one dimension only.

(8) Congenial hosts do not in any way change the morphology of stem-rust spores. A single host plant, common to several biologic forms, lacks the ability to unify them either in size or shape. Nor can several host plants, which are equally susceptible to a single biologic form, exert any influence on the spore morphology of this form.

(9) Resistant host plants and adverse environmental conditions invariably tend to decrease the size of any type of spores of any biologic form. The reduction in size occurs most frequently in spore length only, but sometimes both length and width are affected. As soon as the unfavorable conditions of host and environment are removed, the spores in question will attain their normal dimensions in a single generation.

(10) It is of interest as well as of importance to establish the quantitative measure of the spore shape of the different biologic forms. For this purpose the ratio of length to width should be determined and grouped into classes, the constants calculated and the significance of the differences ascertained.

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PLATE 1

Median longitudinal diagrammatic figures showing comparative differences in the means of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis*.

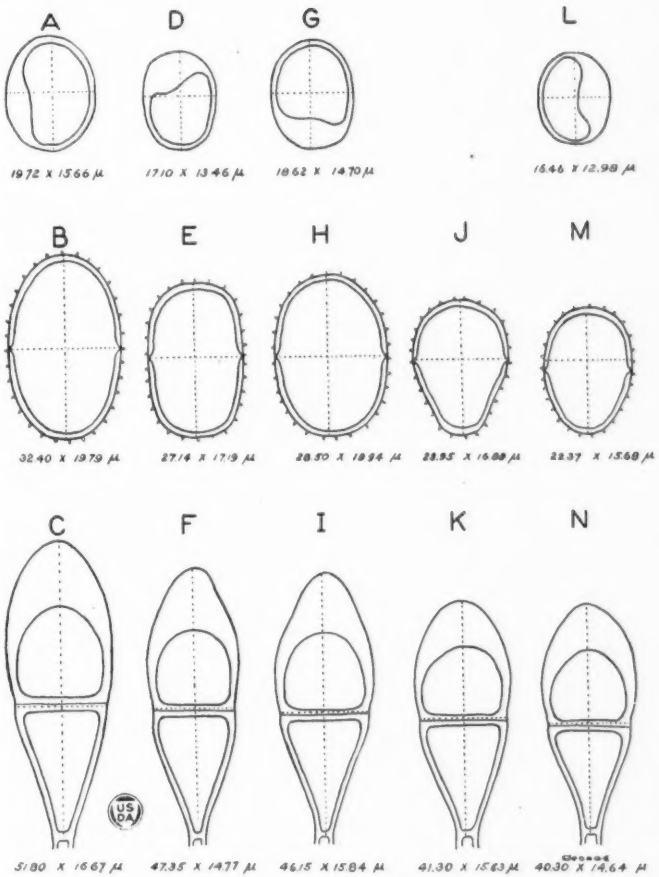
A, B, and C.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis tritici*.

D, E, and F.—Similar diagrams of *P. graminis secalis*.

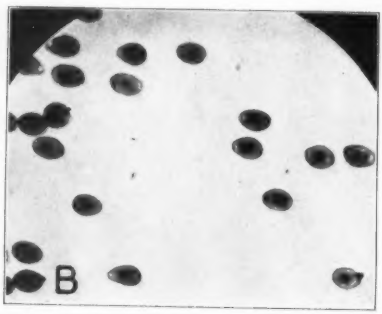
G, H, and I.—Similar diagrams of *P. graminis avenae*.

J and K.—Similar diagrams of urediniospore and teliospore of *P. graminis phleipratensis*.

L, M, and N.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis agrostis*.

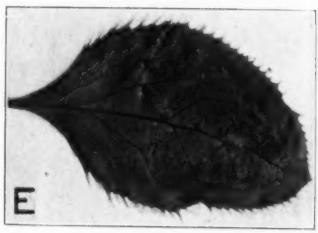

A
D
G
L
19.72 X 15.66 μ
17.10 X 13.46 μ
18.62 X 14.70 μ
16.46 X 12.98 μ
B
E
H
J
M
32.40 X 19.79 μ
27.14 X 17.19 μ
28.50 X 18.94 μ
28.95 X 16.88 μ
22.37 X 15.68 μ
C
F
I
K
N
51.80 X 16.67 μ
47.35 X 14.77 μ
46.15 X 15.84 μ
41.30 X 15.63 μ
40.30 X 14.64 μ

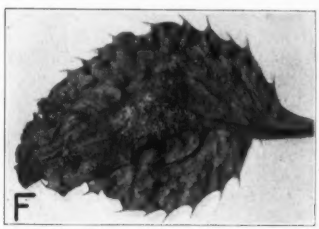
A


B

C

D


E


F

G

PLATE 2

Photomicrographs of uniform magnification showing the relative size and shape of the urediniospores of three biologic forms of *Puccinia graminis:*

A.—*P. graminis tritici.*

B.—*P. graminis phleipratensis.*

C.—*P. graminis agrostis.*

Photographs illustrating the effect of susceptible and resistant barberry species on the aecial development of biologic forms of stem rust:

D and E.—Showing similar and normal development of cluster cups of *P. graminis secalis* on two equally susceptible species, *Berberis vulgaris* and *B. sieboldii*, respectively.

F and G.—Showing the difference in the aecial development of a single biologic form, *P. graminis agrostis*, when grown on susceptible and resistant barberry species, viz, *B. vulgaris* and *B. brevipaniculata*, respectively.

RELATION OF CERTAIN SOIL FACTORS TO THE INFECTION OF OATS BY LOOSE SMUT[1]

By LUCILLE K. BARTHOLOMEW, *formerly Assistant Pathologist*, and EDITH SEYMOUR JONES, *formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture*[2]

INTRODUCTION

In order to interpret properly the results obtained by experimenting with a disease, it is desirable to understand the effect of similar conditions upon the host and fungus apart from each other as well as in combination. Accordingly, an attempt has been made in the present paper to analyze the effects of certain soil factors, first upon the fungus, *Ustilago avenae* (Pers.) Jens., and upon the host, *Avena nuda* L., and then upon the two in combination.

The literature relative to the problem in hand has been adequately reviewed in a related publication[3] by the junior author of this paper (in this issue of the Journal of Agricultural Research), so no further detailed consideration of that feature will be attempted in this paper.

CONDITIONS OF THE EXPERIMENTATION

There are certain environmental factors which must be given thorough consideration in any well-balanced series of pathological investigations. Their relative importance may vary with the host and the fungus under consideration, but their standardization and control, in so far as these are possible, are imperative. Probably the four most important of these factors involved in the present investigations are temperature, moisture, composition of the medium, and its acidic or basic reaction.

The temperatures were regulated very satisfactorily by the use of temperature tanks.[4] The soil moistures were computed and maintained in terms of the saturation point of the soil, which was determined by the commonly accepted standard methods. The soil was sifted through wire gauze into standard 1-centimeter cups, which were leveled off by means of a spatula without tamping, and then were set into a dish of water of known temperature and allowed to stand until the soil was thoroughly saturated. The cups then were removed, drained until no more water dripped from the under surface, and weighed. The soil was dried at a temperature of 100 to 110° C. until it reached a constant weight. From these data the water-holding capacity of the soil was calculated. The standardization of soil water on the basis of dry weight, e. g., 10 gm. of

[1] Accepted for publication Aug. 21, 1922.

[2] The research herein recorded was conducted under the direction of Dr. George M. Reed, formerly pathologist in charge of cereal smut investigations, Bureau of Plant Industry, Washington, D. C., but now at the Brooklyn Botanic Gardens. We wish to extend our grateful thanks to him as well as to Dr. L. R. Jones and his associates in the Department of Plant Pathology, University of Wisconsin, for their hearty cooperation at all times.

This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[3] JONES, Edith Seymour. INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF USTILAGO AVENAE. *In* Jour. Agr. Research, v. 24, p. 577–591, 3 fig. Literature cited, p. 590–591.

[4] JONES, L. R. SOIL TEMPERATURES AS A FACTOR IN PHYTOPATHOLOGY. *In* Plant World, v. 20, p. 229–237. 1917. Literature cited, p. 236–237.

water to 100 gm. of dry soil, was discarded after trial because of the variation in soil-moisture conditions when soils of different water-holding capacities were used. The chief danger of the soil-saturation method lies in the unequal compacting of the soil but this error may be minimized by using a uniform method for handling all soil samples.

The soil moistures were held constant by the use of galvanized containers, insulated soil surfaces, and daily weighings for the determination of any moisture lost through evaporation. The soil reaction was determined by the Truog[5] method and was found in all cases to be "medium acid." The soil composition offered the greatest difficulties in the matter of uniformity, but these were overcome by securing soil from approximately the same locality for all the series of experiments. It was a sandy loam with a water-holding capacity of 28 to 30 per cent for the first year's experiments and 39 to 41 per cent for those of the second year. Pure-line seed of the previous season was used, and sowings were made at a depth of 1 inch. The soil was thoroughly sifted, poured into the can, and tamped by dropping the can from a given height a uniform number of times, thus obtaining a fairly constant state of compactness.

INFLUENCE OF TEMPERATURE ON GROWTH OF FUNGUS AND HOST

The fungus, *Ustilago avenae* (Pers.) Jens., was obtained from smutted heads of *Avena nuda* L. grown at Columbia, Mo., in the summer of 1918. This material was used for all of the inoculations during the winter and spring of 1918–19. Smutted heads obtained from our own plats of *A. nuda* L. in the summer of 1919 furnished the inoculum for the following year. Pure-line seed of *A. nuda* L., the hulless or naked oat, was chosen as the standard for experimentation because this species shows a high degree of susceptibility to loose smut. Uniform moistures were maintained throughout the infection period and for one week afterwards, following which the containers were removed from the tanks. The plants taken from the tanks during the colder months were allowed to mature in the containers in which they were growing, but those in the series removed from the tanks in the spring months were transplanted at once to open plats and allowed to mature out of doors.

The study of the fungus in culture has been limited, but the results are clear cut. Potato-dextrose agar, with a reaction of +10 on the Fuller scale, was used. Agar sticks were melted, cooled, and heavily inoculated with sporidia from pure cultures of the fungus. These were poured into plates and allowed to incubate until many colonies appeared. Series of plates of the same medium then were inoculated by selecting uniform colonies and transplanting them. The single-spore method of culturing the organism was found impracticable because of the minute size of the spores, their tendency to cling together, and their poor germination, even when incubated under optimum conditions. The results obtained are shown in Table I.

When the cultures were removed from the Altmann incubators at the end of six days and allowed to remain at room temperature, growth occurred in all except those which had been exposed to 36° C. Growth, however, was very slight in cultures taken from 32°, showing that only a small percentage of the sporidia were able to survive exposure to this temperature. This was not due to desiccation of the medium, because sufficiently high humidity was maintained to prevent undesirable evapo-

[5] TRUOG, E. A NEW TEST FOR SOIL ACIDITY. Wis. Agr. Exp. Sta. Bul. 249, 16 p., 3 fig., 1 col. chart. 1915.

ration. Low temperatures appeared to inhibit growth without causing any permanent injury to the fungus. Cultures were kept at the low temperatures in some cases for a period of four weeks or more, but no growth occurred. When these same cultures were transferred to warmer temperatures, growth soon was evident.

TABLE I.—*Effect of temperature on growth of Ustilago avenae in culture*

Average temperature.	Amount of growth.
C.	
5°	None.
8°	Very slight.
14°	Slight.
16°	Fair.
20°	Good.
23°	Fair.
28°	Slight.
32°	Very slight (if any).
36°	None.

EFFECT OF TEMPERATURE ON GERMINATION OF SPORES AND PRODUCTION OF SPORIDIA

The spores of *Ustilago avenae* placed in Van Tieghem cells in beef broth with a reaction of +10, Fuller's scale, germinated between 5° and 34° C., the minimum being between 4° and 5°, the optimum between 15° and 28°, and the maximum between 31° and 34° C. For sporidial production under the same conditions of culture as for spore germination the minimum and optimum are the same as for spore germination, but the maximum, 30°, is lower.

THE EFFECT OF TEMPERATURE ON GERMINATION OF THE HOST, AVENA NUDA L.

It is difficult to estimate the growth of the host as it is not easy to define a standard for growth. The plants in culture, 1 to 2 months old, appeared to be most vigorous where germination and growth of the seedlings had occurred at temperatures of 16° to 24° C. They elongated more rapidly at the higher temperatures but presented an etiolated appearance as compared with the healthy, stalky growth at the lower temperatures. The record is given in Table II.

TABLE II.—*Effect of temperature on germination of Avena nuda L.*

Average temperature.	Observations on growth.
C.	
3°	Germination but no subsequent growth.
5°	Germination but no subsequent growth.
7°	Germination with slight growth.
10°	Germination with slow growth.
15°	Secondary roots produced, plumule 1.3 cm. long.
19°	Optimum growth, plumule 3 cm. long.
21°	Good growth, plumule 2 cm. long.
27–33°	Less vigorous, plumule 1 cm. long.

Table II gives a fair idea of the effect of the range of temperature, 3° to 33° C., upon germination and seedling growth. The optimum lies at an average temperature of 19° C.

If we compare the host and fungus in their temperature relations in the light of the above data, we find a rather close correlation. The fungus shows a tolerance for somewhat lower temperatures, especially as to germination and initial production of sporidia (essential to infection), while the host shows a tolerance for higher temperatures. However, their optima for germination and growth are practically identical.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION

In order to visualize quickly this phase of the problem, it may be well to review briefly the salient points in the life history of the fungus. The spores of *Ustilago avenae*, the loose smut of oats, are for the most part seed-borne. When the oat seed is sown the spores germinate, producing masses of sporidia which continue to bud and multiply much like yeast. As the oat kernel germinates, the sporidia send out germ tubes and infect the young seedling through the coleoptile. By the end of 10 days or 2 weeks the mycelium penetrates through the coleoptile and subsequently establishes itself in the meristematic tissue of the growing point. According to various investigators, infection is limited to the first few days following germination of the oat kernel and none occurs after the growing point emerges from the coleoptile.

As the plant develops, the fungus is carried upward in the growing point of the primary shoot, or of the lateral shoots. Previous to the time of heading, it is impossible to see any difference between smut-free and infected plants, except for a slight lagging in vegetative growth, which is not a safe diagnostic feature. At heading time, however, the fungus appears to gain the ascendency and the mycelium spreads rapidly through the young ovaries, in which are produced the masses of black spores. At times the fungus appears in linear pustules on the leaves, resembling leaf smut of various grasses, and very often the glumes are striated with the black spore masses. The histological features of the fungus have been adequately described by Lutman.[6]

Obviously the problem in hand is concerned with soil infestation and the physical and chemical conditions of the soil at the time of infection of the oat seedling. Subsequent atmospheric conditions may influence the vegetative growth of the host and thus determine the ability of the fungus to maintain its position in a rapidly dividing meristematic tissue. It is interesting to note in this connection, however, that the most vigorously growing plants appear to be the most heavily smutted. Just why two heads on one plant may be entirely smutted and three other heads on the same plant remain free from smut, or conversely, offers an interesting problem of disease escape. Furthermore, it is not uncommon to see a single spikelet of a single head smutted and all the rest of the head smut-free.

As indicated by the three preceding paragraphs, soil temperature and soil moisture may have a marked influence on infection. Consequently, numerous series of experiments were performed in order to determine the influence of these two factors upon infection of the host by the fungus. The data secured are presented in Tables III and IV.

[6] LUTMAN, B. F. SOME CONTRIBUTIONS TO THE LIFE HISTORY AND CYTOLOGY OF THE SMUTS. *In* Trans. Wis. Acad. Sci., v. 16, p. 1191–1244, pl. 88–95. 1910. Literature cited, p. 1225–1228.

A uniform soil moisture of 36 per cent (equivalent to 10 grams of water to 100 grams of dry soil with a soil saturation point of 27.8 per cent) was maintained throughout the experiments from which the data in Table III were obtained.

TABLE III.—*Effect of different soil temperatures on infection of Avena nuda by Ustilago avenae, when soil moisture was maintained at 36 per cent*

Average temperature.	Dry seed.		Soaked seed.	
	Clean plants.	Smutted plants.	Clean plants.	Smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8°	33	67	20	80
12°	50	50	10	90
15°	0	100	0	100
18°	0	100	0	100
21°	0	100	0	100
23°	0	100	25	75
26°	33	67	8	92
29°	25	75	89	11

A soil moisture of 67 per cent (equivalent to 19 grams of water to 100 grams of dry soil) with a soil saturation point of 27.8 per cent was maintained throughout the experiment, the results of which are shown in Table IV.

A comparison of Tables III and IV shows soil temperature to be a limiting factor in infection. The extent of its importance, however, varies with the amount of water present in soil or seed.

TABLE IV.—*Effect of different soil temperatures on infection of Avena nuda by Ustilago avenae when soil moisture was maintained at 67 per cent*

Average temperature.	Dry seed.		Soaked seed.	
	Smut-free plants.	Smutted plants.	Smut-free plants.	Smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
9°	100	0	91	9
12°	47	53	63	37
16°	13	87	72	28
21°	17	83	71	29
26°	39	61	67	33
29°	94	6	100	0

The results given in Table V are based upon the totals secured from a large number of series of experiments and are based on a total of 1,489 plants. The soil moistures are indicated in the table, and the soil-saturation point varies from 39 to 41 per cent. Air-dry seeds were used throughout.

The totals under 80 per cent soil moisture are not based on results obtained from as large a number of plants as in either of the other moisture tests. The high soil moisture was unfavorable for the germination of the grain and many series were reduced or failed utterly as a result, but the percentages given were based on a sufficiently large number to justify inclusion in the results.

TABLE V.—*Effect of soil temperature and soil moisture on infection of Avena nuda by Ustilago avenae*

Average temperature.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10°–14°	30–35	96. 5	60	74. 0	80	56. 0
14°–16°	30–35	96. 5	60	58. 5	80	1. 0
17°–21°	30–35	98. 0	.....	.....	.....	.....
18°–22°	30–35	100. 0	60	93. 0	(Series failed)	
31°–32°	30–35	24. 0	60	0	80	0

When Table V is compared with Tables III and IV, it will be noted that a less pronounced temperature relationship is evidenced, especially at the lower moisture contents. At a temperature of 31° to 32° C. there is a decided falling off in infection at all soil moistures. These results indicate that one would be safe in predicting that high soil temperatures tend to limit infection regardless of soil moistures. When we consider that 32° C. is the maximum temperature for germination and growth of the fungus, we would expect to find this decrease in infection, especially when we consider the rapidity of germination and elongation of the young seedlings at this temperature.

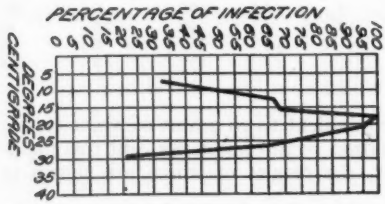


FIG. 1.—Influence of soil temperature upon the infection of *Avena nuda* by *Ustilago avenae* when soil moisture is disregarded.

It will be noted that a temperature of 18° to 22° C. furnishes the optimum for infection in all soil moistures. This coincides directly with the optimum for germination and growth of both host and fungus.

The high percentage of infection at 10° to 14° C. might correlate with the longer time the seed remains in the ground, especially in the case of the lower soil moistures. It may require as long as three weeks under these conditions for the seedling to appear above ground. In this connection, experiments on the relation of soil moisture to the germination of the spores show that the spores germinate as readily in a soil moisture of 35 per cent as in a soil moisture of 65 per cent. If this is correlated with the temperature data already cited, it will be seen that there is no reason why the fungus might not become fairly well established in the soil while the seed remains dormant, or very slowly germinating.

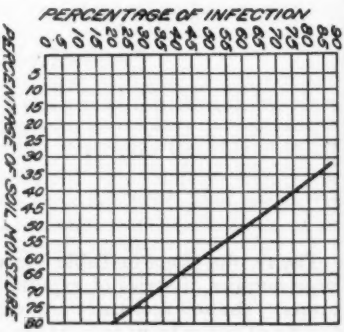


FIG. 2.—The influence of soil moisture upon infection when soil temperature is disregarded.

Such a curve as is shown in figure 1 may help to interpret the common observation that the early sowing of oats in the spring tends to

reduce the amount of infection by loose smut. On the other hand, fall sowing, while the soil temperature is high, would tend also to cut down infection. Such a curve corresponds more nearly to the actual conditions obtaining in nature than a curve derived from series wherein the various factors are considered separately.

The graph in Figure 2 again serves to interpret what is found under field conditions. Conditions of high moisture combined with low temperatures in the spring undoubtedly would tend materially to reduce infection. This conclusion was borne out by some field experiments. Plats containing 28,000 heavily inoculated seeds of *Avena nuda* were prepared in April and May of 1919. The spring was very wet and cold. The average soil temperature was 11.1° C. with a range from 2° to 24° C. during the germination period. The soil moisture averaged from 45 to 50 per cent of the soil saturation point, and ranged as high as 70 per cent at times. The plants matured normally but contained less than one-tenth of 1 per cent of smutted plants. Seed from the same lot treated with the same inoculum, but grown under controlled conditions favorable to maximum infection, produced 100 per cent of smutted plants.

SUMMARY

(1) The temperature range for growth of *Ustilago avenae* when cultured on potato-dextrose agar with a +10 reaction is, minimum 8° C.; optimum 20° C.; maximum 32° C.

(2) The temperature range for germination of the spores in beef broth with a +10 reaction is, minimum 5° C.; optimum 15° to 28° C.; maximum 31° to 34° C.

(3) The temperature range for sporidial production under the same conditions as for spore germination is, minimum 5° C.; optimum 15° to 28° C.; maximum 30° C.

(4) The temperature range for germination of the seeds of *Avena nuda* is, minimum 3° C. with no measurable subsequent growth; optimum 18° to 20° C.; maximum 33° C. plus, not definitely established.

(5) The temperature at which the seed is germinated and the seedlings maintained for two weeks appears to influence the subsequent vigor of the plants. The most vigorous plants were secured from seedlings which had been kept at 16° to 24° C.

(6) High soil temperatures were accompanied by a marked reduction in the percentage of infection under the conditions of our experiments.

(7) Low soil temperatures also were accompanied by a reduction in the percentages of infection, but to a much less marked degree than in the case of high temperatures.

(8) High soil moistures, combined with high soil temperatures, result in complete elimination of the fungus.

(9) Low soil moistures, within a certain temperature range, are accompanied by relatively high percentages of smut infection.

INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF USTILAGO AVENAE [1]

By EDITH SEYMOUR JONES, *Formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The relation of climatic factors to the development of the smuts has been a matter of speculation during rather more than two decades. An exact determination of the influence of these factors has recently been undertaken by Dr. Lucille K. Bartholomew and the writer (*2*),[2] in order to provide experimental conditions for the determination of the smut resistance of certain pure lines of species of Avena. It has been pointed out in that paper that in order to decide whether any given variety be completely resistant, its performance under conditions resulting in 100 per cent of smut infection in susceptible plants must be ascertained. In determining those factors most favorable to the development of the smut organism, it becomes manifest that these same factors are numerous and closely interwoven, affecting both the host and the fungus. Their effect on both the host and the fungus must be distinguished if a complete analysis of their relation to the development of the disease is to be achieved.

While Doctor Bartholomew and the writer were determining the effect of temperature and moisture (*2*) upon infection by *Ustilago avenae* (Pers.) Jens. (*5*), the writer began to study the influence of temperature upon the germination of the spores of this fungus. When the results of the infection studies began to indicate that soil moisture is a factor controlling infection, the effect of this factor upon the germination of the spores was studied, and later the necessity of studying the relation of oxygen to spore germination became evident. This paper presents the experimental evidence which has been obtained concerning the influence of these three factors upon the germination of the spores of *Ustilago avenae*. Most of these experiments were performed under the guidance and with the help of Doctor Bartholomew, who had carefully formulated the whole problem in its broader aspects.

By way of further introduction to the review of literature and the experiments, it may be well to state briefly the life history of the fungus in its relation to the infection of the host plant. The spores of *Ustilago avenae* overwinter mainly on the seed. The spores germinate simultaneously with the oat seed. They rarely form germ tubes but commonly produce promycelia which, in turn, form either sporidia or thin hyphæ. These sporidia, hyphæ, or germ tubes infect the host through the coleoptile before it is pierced by the first leaf and the fungus establishes itself in the meristematic tissue of the growing point of the main axis and the tillers. Here the mycelium keeps pace with the development of the host, without apparent injury to it, until, at blossoming time, it

[1] Accepted for publication Aug. 21, 1923. This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[2] Reference is by number (italic) to "Literature cited," p. 590-591.

produces its chlamydospores in the young ovaries. Many of these spores, when scattered, come in contact with the seed and the life cycle is repeated.

REVIEW OF LITERATURE

RECOGNITION OF THE RELATION OF CLIMATE TO THE SMUTS

The first recognition of a relation between environment and the development of the smuts of oats, or, in fact, of any closely related smut, was made by Jones (*15*). The observations recorded in that publication indicated, in his opinion, that certain climatic conditions prevailed in Vermont which were especially unfavorable for the development of oat smut. In the following year, however, Jones (*16*) found a higher percentage of smut in Vermont than in other sections of the country. These observations were supplemented by the records, for one season, of the soil temperature during the period of germination and early development of the seedling. From these accumulated data, the writer concludes—

> it is evident that surrounding climatic or soil conditions may at any point in the contest so favor one or the other of the contestants—either the oat plant or the smut fungus—as to decide which shall come out victorious.

ANALYSIS OF THE RELATION OF TEMPERATURE TO THE SMUTS

Following Jones, other workers have recognized soil temperature as a factor of primary importance. Among them Hecke (*12*) distinguishes a threefold effect of temperature, involving particularly (1) the germination of the spores and seeds, (2) the duration of conditions in host plant favorable for infection, and (3) the possibility of the fungus reaching the growing point of the plant. The greater number of the other publications dealing with the relation of temperature to the disease can be classified according to the aspects thus distinguished.

EFFECT OF TEMPERATURE UPON GERMINATION OF SPORES AND SEEDS

Herzberg (*13*) found the cardinal points of temperature for spore germination of *Ustilago avenae* and of four other smuts to be as follows: Minimum between 5° and 11° C., optimum 22° to 30°, and maximum 30° to 35°.

Ravn (*20*) very early noted that the amount of oat smut varies with the time of sowing oats. Later, detailed experiments made possible the definite statement that the time of sowing affects greatly the occurrence of oat smut, that the quantity of smut is small or negligible when oats are sown early, and that it increases in later sowings. A decrease with very late sowing was observed in a few cases. In his discussion of the possible reasons for this climatic influence he presumes that the temperature during germination is the most important factor.

Tubeuf (*22*), experimenting with the smut of oats, found no infection when the temperature was below 7° C. during the seedling stage and 20 to 24 per cent at a temperature of 20° to 21°. In accounting for this difference, he showed that the minimum for the germination of the oat plant is lower than that of the smut, so that when the temperature is sufficiently low the host will escape infection. Thus, he is led to recommend early sowing. In a short paper, Appel and Gassner (*1*) make some general remarks on oat smut, which are essentially in agreement

with the views of Tubeuf (*22*), as given in the first of this paragraph. Also Eriksson (7) considers that damp, warm weather during the sowing favors infection. Heald and Woolman (*10*) have found that a range of temperature between 40° and 65° F. during the germination of wheat is more favorable for bunt infection than either a higher or a lower temperature. It is of interest that this range includes the minimum and the optimum for the germination of bunt spores as given by Hecke (*12*). There are several nontechnical bulletins which mention the importance of temperature. Heald (*9*), in advising the early or late sowing of wheat, shows that during August not only is there a comparatively small number of smut spores in the soil, but that also the soil temperature is generally too high for optimum infection; and by the end of October or the first of November, not only has much of the wind-blown smut germinated, but also there is the frequent occurrence of a soil temperature too low for ready infection. In a similar publication, Heald and Zundel (*11*) state that early seeding of oats results in the minimum amount of smut.

DURATION OF SUSCEPTIBILITY OF HOST PLANT TO INFECTION

Volkart (*23*) was the first to emphasize the fact that low temperature may prolong the infection period. He states that with oats and wheat the slower the initial stages of growth, the more abundant and severe will be the infection. However, extremely low temperatures may retard not only the germination of the seed but also may retard even more the germination of the smut spores and thus remove the danger of infection. This point is more clearly set forth by Hecke (*12*). The latter's experiments dealt with the bunt of wheat which he found occurred most abundantly among plants which had passed their early stages during the low temperatures of late fall and early spring. The temperature of the first five days seemed decisive. That low temperatures at that time caused increased infection he lays in the main to the prolonging of the infection period, although he also discusses what he considers a greater ease with which the fungus may reach the growing point. In comparing oat smut with bunt in this temperature relation, he considers the low temperature not only as a factor retarding the development of the oat seedling, but also as one delaying the germination of the smut spore. The possibility of this being true is substantiated by the cardinal points of temperature. These, according to his citations, are as follows: For oats, minimum 4° to 5° C., optimum 25°, and maximum 30°; for oat smut, minimum 5° to 11° (9°), optimum 22° to 30°, maximum 30° to 35°; for wheat, minimum 3° to 4.5°, optimum 25°, maximum 30° to 32°; and for bunt spores, minimum less than 5°, optimum 16° to 18°, maximum less than 25°.

Munerati (*19*) emphasizes the importance of the duration of the infection period for heavy bunt infection. He states:

> The faster the first phases in the evolution of the life of the seedling are passed through, the more completely is it able to escape attack by Tilletia, and vice versa.

He comes to such conclusions as the result of experiments recorded in two publications (*18*, *19*).

It is obvious that a deeper sowing of seeds might have the effect of prolonging the infection period in a way similar to a low temperature. Jones (*16*) makes note of the factor of the depth of seeding.

Kirchner's (*17*) conception is at variance with that of Volkart (*23*), Hecke (*12*), and Munerati (*19*). Kirchner, also working with bunt, says

there is no connection between the ability of the young plant to push through the ground and the attack of smut. He considers the susceptibility of the host to be dependent upon internal chemical differences, and that the host is not affected by external conditions so as to permit the entrance or bring about exclusion of the smut.

POSSIBLE EFFECT OF TEMPERATURE ON THE ABILITY OF THE FUNGUS TO REACH THE GROWING POINT OF THE HOST AFTER PENETRATION

Hecke's third aspect, the possibility of the fungus reaching the growing point of the host plant, is difficult to distinguish from the second, the prolongation of the infection period. Investigators dealing with the disease do not know without studying the host and the fungus separately which of these is modified by the temperature factor. Hecke classes Brefeld's (*3*) work under the third aspect in which the fungus is considered modified. Using germinated oat smut spores as inoculum, with a lower temperature (one not over 7° C.) he obtained 40 to 46 per cent infection, whereas at a higher temperature (above 15° C.) he obtained 27 to 30 per cent. With the factor of the germination of the smut spores eliminated, Hecke concluded that the fungus reached the growing point of the host more easily at the lower than at the higher temperature. From his results, Brefeld considers that if, in cultivation, the seeding is done in warm weather, followed by cold, more smut will develop than when the temperature remains constantly high. The use of germinated spores as inoculum makes it impossible to compare Brefeld's work with that of other investigators of oat smut.

RELATION OF MOISTURE TO THE SMUTS

The relation of moisture is discussed by Clinton (*4*) and by Hungerford and Wade (*14*). The former notes greater loss from both loose and covered smut of oats in the "hardpan region" of Illinois than in central Illinois. He thinks that the higher moisture content of the soil in this region favors the fungus and causes more infection. The latter observed that in fields sown immediately after rain, a higher percentage of bunt appeared than in those sown under relatively dry conditions. Results obtained from their greenhouse experiments seemed to confirm this observation.

EXPERIMENTAL STUDIES

THE INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The first problem was to redetermine the cardinal points of temperature for spore germination. The spore material used was grown the spring previous to experimentation and was from 2 to 7 months old when used. To insure its uniformity, all the smutted heads were kept in one envelop, and the spores shaken from the heads were introduced into 30 cc. of medium in approximately the same quantities each time. After trying several different media, a beef broth (4 gm. Liebig beef extract and 10 gm. peptone per 1,000 cc. of distilled water) of +10 Fuller's scale ($P_H 6.1$) was chosen. About two small loopfuls of the spore suspension were mounted in Van Tieghem cells or hollow-ground slides. As Duggar (*6*) has shown the form of the drop and the amount of evaporation to be significant, care was taken that all conditions of mounting

should be as uniform as possible. Duplicate mounts were placed at each temperature, and the experiment was replicated 15 times. Automatically regulated incubators furnished the constant temperatures to which the spores were subjected. At each observation the percentage of germination of 400 spores was recorded for each cell.

The results of a typical experiment are expressed in Table I, and Figures 1 and 2. The results of all the experiments are brought together in Table II.

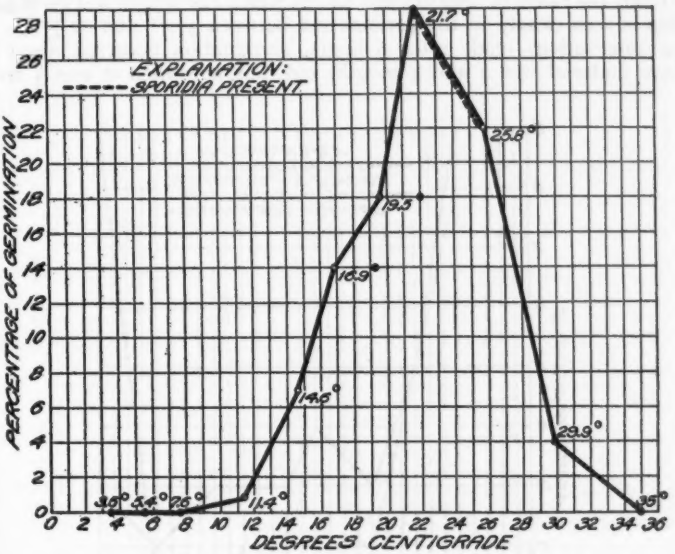


FIG. 1.—Average percentage of germination, and presence of sporidia, of *Ustilago avenae*, in 24 hours, as influenced by temperature of incubation, in experiment 5.

TABLE I.—*Average percentage of germination of chlamydospores of Ustilago avenae produced 5 months earlier, incubated at different temperatures, and recorded at successive intervals*

Temperature average.	Temperature range.	Time.									
		¼	1	2	3	4	5	6	7	8	9
C.	*C.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
3.5°	2.6°– 4.8°	0	0	0	0	0	0	0	0	0	0
5.4°	4.4°– 6.0°	0	0	0	0	0	.4	2	3	8	10
7.6°	6.4°– 8.8°	0	0	0	2	14	18.0	....	....	....	....
11.4°	10.0°–12.5°	0	.7	10	27	27	.....	....	....	....	....
14.6°	13.6°–15.9°	0	7.0	22	27	34	.....	....	....	....	....
16.9°	16.1°–18.5°	0	14.0	21	32	....	.....	....	....	....	....
19.5°	17.8°–20.6°	.7	18.0	27	27	.....	.....	....	....	....	....
21.7°	19.7°–23.5°	3.0	29.0	27	31	....	.....	....	....	....	....
25.8°	22.5°–28.4°	1.3	22.0	33	....	....	.....	....	....	....	....
29.9°	28.4°–31.4°	.5	4.0	2	5	3	.....	....	....	....	....
35.0°	34.0°–36.0°	0	0	0	0	0	.....	....	....	....	....

In order that Table II be clear to the reader, there are four facts which must be borne in mind. First, in many cases the incubators did not hold the temperatures within satisfactory limits, so that the upper limit of the temperature range was more significant than the average temperature, especially when the spores were incubated for a long time. For this reason the minimum temperature for spore germination rests between 4° and 5° C. In the second place, the age of the spore material influences very markedly the percentage of germination and, to a small degree, extends the range of temperature within which the spores will germinate. This is seen clearly in the germination between 31.7° and 32.7° C. Of six observations made after 6 hours, germination occurred when the spore material was 2 and 4 months old, but not when it was 5 and 6

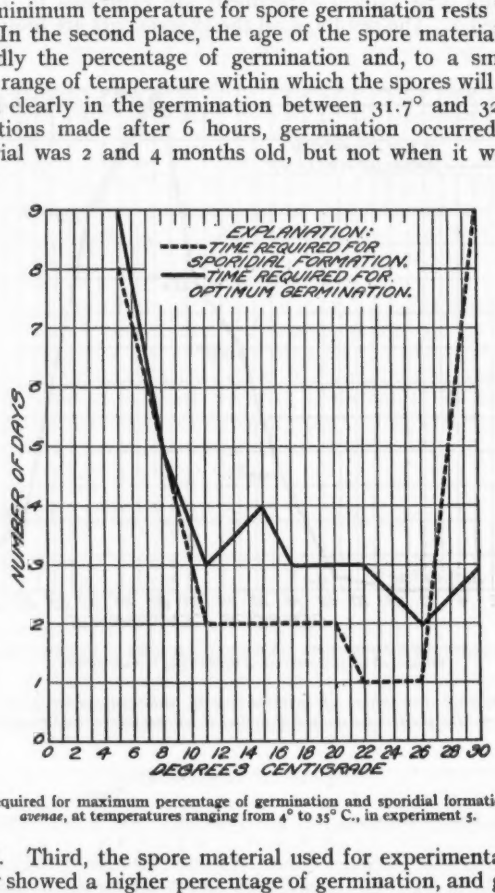

EXPLANATION:
TIME REQUIRED FOR SPORIDIAL FORMATION.
TIME REQUIRED FOR OPTIMUM GERMINATION.
NUMBER OF DAYS
0 1 2 3 4 5 6 7 8 9
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
DEGREES CENTIGRADE

FIG. 2.—Time required for maximum percentage of germination and sporidial formation with *Ustilago avenae*, at temperatures ranging from 4° to 35° C., in experiment 5.

months old. Third, the spore material used for experimentation in the second year showed a higher percentage of germination, and germination at a wider range of temperatures than that used the first year. This accounts for the fact that experiments 11 to 15, inclusive, conducted during the second year, show higher percentages than the earlier experiments. In the fourth place, germination apparently decreased after a certain length of time, as some of the figures in the table show. This, of course, could not be due to an actual decrease, but was due to the fact that the promycelia broke away from some of the spores, leaving them indistinguishable from ungerminated spores. The examinations were discontinued in most cases when lower percentages were obtained. Where the records were made, however, the figures were recorded to show in a more decisive way where the maximum germination was attained.

TABLE II.—*Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germina-*

TABLE II.—*Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germination of the chlamydospores of Ustilago avenae of different ages, incubated at different temperatures, and recorded at successive intervals.*

Experiment No.	Age of spores.	Average temperature.	Temperature range.	Time in days.																		
				¼	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	28
	Months.	*C.*	*C.*																			
8	6	2.9°	2.4°–3.8°	0	.....	.....	0	0	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	.....	0	.....
9	7	2.9°	2.4°–3.8°	.....	.....	.....	.....	0	.....	.....	.....	0	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....
12	2	3.5°	2.8°–4.0°	0	0	0	0	0	0.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	3.5°	2.6°–4.8°	0	0	0	0	0	0	0	0	0	0	0	0.04	0	.....	.....	.....	.....	.....	.....	.....
7	6	3.5°	2.9°–4.4°	0	0	0	.....	0	.....	0	.....	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	5	3.6°	3.1°–4.2°	0	0	0	0	.....	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	3.7°	3.2°–4.3°	0	0	0	0	.3	2.0	7.0	17.0	11.0	25.0	29.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	3.9°	2.8°–4.7°	.....	0	0	0	.....	.....	.05	.1	.5	1.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	4.1°	3.2°–5.0°	.....	.....	.....	.....	.....	.04	.06	.27	5.0	8.0	7.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	4.2°	1.8°–5.4°	.....	.....	.....	0	0	0	.....	0	0	.....	0	0	0	.....	0	0	0	0	.....	.....
15	5	4.2°	3.6°–5.1°	.....	.....	.....	0	0	0	.....	.03	.3	.....	.....	.4	.....	.....	.....	.6	.....	.9	.8	3
8	6	4.5°	3.6°–5.4°	0	.....	.....	0	0	.....	0	.....	.....	.07	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	4.6°	3.6°–6.3°	0	0	0	.....	0	.....	0	.....	0	.....	.....	.04	.....	.....	.....	.....	.....	.....	.....	.....
15	5	4.8°	3.7°–6.0°	.....	.....	.....	.02	2.0	4.0	.....	8.0	8.8	.....	.....	9.4	14.0	.....	.....	.....	.....	.....	.....	.....
9	7	4.8°	4.0°–5.4°	.....	.....	.....	.....	.....	.....	.....	.....	.5	.....	.....	.....	.2	.....	.....	.....	.....	.....	.....	.....
6	5	5.2°	4.2°–6.6°	0	0	0	0	.....	.04	.13	.13	.....	.....	.....	.....	.....	.....	1.4	.....	4.0	10.7	.....	.....
5	5	5.4°	4.1°–6.0°	0	0	0	0	0	.4	2.0	3.0	8.0	10.0	9.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	5.5°	4.2°–6.4°	0	0	0	.03	.03	.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	5.6°	4.3°–6.9°	.....	.....	.....	.....	.21	16.0	39.0	.....	44.0	53.9	43.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	5.9°	4.8°–6.8°	.....	.....	0	5.0	.....	13.0	20.0	20.0	18.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	6.4°	4.4°–7.4°	0	0	0	.....	2.0	.....	5.0	.....	14.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	7.6	6.4°–8.8°	0	0	0	2.0	14.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	8.0°	7.2°–8.3°	.....	0	2.0	.....	.....	5.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	10.4°	9.2°–11.0°	.....	.13	2.0	.....	.....	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	11.4°	10.0°–12.5°	0	.7	10.0	27.0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	12.8°	11.0°–15.6°	0	14.0	27.0	33.0	46.0	36.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	13.9°	12.0°–17.3°	0	19.0	26.0	37.0	38.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	14.6°	13.6°–15.9°	0	7.0	22.0	27.0	34.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	16.5°	16.0°–17.3°	0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	16.7°	15.7°–18.8°	.03	58.0	67.0	67.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	16.9°	16.1°–18.5°	0	14.0	21.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	17.1°	16.2°–18.0°	0	17.0	21.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	18.2°	17.2°–20.0°	2.3	59.0	66.0	66.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	18.4°	18.0°–19.2°	.07	21.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	18.6°	17.2°–19.9°	3.1	42.0	40.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	19.1°	17.0°–20.2°	.3	18.0	.....	19.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	19.5°	17.8°–20.6°	.7	18.0	27.0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	19.9°	19.2°–20.0°	.05	16.0	19.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	21.6°	20.3°–23.9°	3.8	53.0	57.0	58.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	21.7°	19.7°–23.5°	2.7	29.0	27.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE II.—*Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germination of the chlamydospores of Ustilago avenae of different ages, incubated at different temperatures, and recorded at successive intervals*—Continued.

Experiment No.	Age of spores.	Average temperature.	Temperature range.	Time in days.																			
				¼	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	28
	Months.	*C.*	*C.*																				
12	2	22.5°	21.5°–23.5°	5.6	31.0	30.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	22.5°	21.5°–26.5°	9.3	21.0	24.0	27.0	13.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	22.6°	21.5°–24.0°	8.2	64.0	74.0	59.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	24.2°	24.0°–24.5°	3.8	14.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	24.6°	23.4°–25.6°	14.0	34.0	35.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	2	24.8°	23.5°–25.8°	16.0	49.0	35.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	25.5°	25.3°–25.8°	5.0	18.0	22.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	25.8°	22.5°–28.4°	1.3	22.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	26.4°	26.2°–27.0°	4.0	13.0	13.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	26.9°	26.0°–28.0°	11.0	43.0	37.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	27.5°	26.9°–28.3°	9.8	23.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	28.1°	27.3°–28.3°	14.0	54.0	62.0	60.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	28.2°	28.0°–28.3°	5.0	27.0	32.0	34.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	28.5°	27.8°–29.0°	3.7	53.0	48.0	43.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	29.1°	27.8°–29.8°	.5	12.0	8.5	.....	9.2	.....	8.5	.....	.....	11.0	.....	10.0	.....	.....	8.2	.....	.....	8.5	.....	.....
6	5	29.1°	29.4°–30.0°	2.2	16.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	29.7°	29.3°–30.3°	6.0	20.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	29.8°	29.6°–30.1°	2.0	15.0	17.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	29.9°	29.6°–30.6°	.....	.1	.....	.....	4.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	29.9°	28.4°–31.4°	.5	4.1	2.0	5.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	6	30.0°	29.4°–30.8°	0	7.0	8.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	2	30.1°	30.0°–30.3°	.3	7.8	9.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	30.2°	29.7°–31.7°	.....	.1	.2	.4	.15	.35	1.5	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	30.4°	29.9°–31.0°	.9	6.0	2.5	4.7	3.6	.5	.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	31.1°	31.0°–32.0°	.4	5.0	12.0	6.0	6.1	4.5	5.3	3.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	31.9°	31.0°–32.2°	.06	.27	.6	.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	32.2°	31.8°–32.7°	.02	.35	.35	1.7	.5	1.7	.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	32.3°	29.3°–34.2°	0	0	.5	.....	.....	.....	.3	.....	.....	.4	.....	.....	.3	.....	.....	.....	.....	.4	.....	.....
8	6	32.4°	31.7°–33.9°	0	4.0	.....	6.0	.....	.....	.....	.....	.....	.....	4.3	.....	.....	.....	.....	.....	2.1	.....	.....	.....
6	5	32.7°	32.4°–33.2°	0	0	.2	.2	.....	.15	.06	.11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	32.9°	31.7°–33.9°	.....	2.0	.....	.....	3.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	33.6°	33.2°–35.0°	.....	0	.....	.....	0	.....	.....	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	.....
12	2	34.1°	33.2°–35.0°	0	0	.015	.01	.015	.025	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	34.3°	34.0°–35.0°	0	0	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	34.4°	33.8°–35.2°	.....	.....	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	34.7°	34.2°–35.2°	0	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	6	34.7°	34.0°–35.5°	0	0	.....	0	0	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	0	.....	0	.....
7	6	34.8°	33.4°–39.5°	0	0	0	.....	0	.....	0	.....	0	.....	.....	0	.....	.....	0	.....	.....	0	.....	.....
5	5	35.0°	34.0°–36.0°	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	35.2°	35.0°–35.6°	.....	0	0	0	.....	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

With these three considerations in mind, it may be concluded that the spores begin to germinate between 4° and 5° C., and that the maximum temperature for germination lies between 31° and 34°. If the optimum be arbitrarily chosen as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours, it is 15° to 28°. The minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from 29° to 30°.

A few interesting observations may be noted here. In the broth solution the spores most commonly germinate into promycelia from which sporidia are cut off, in contrast with the germination on agar placed in the soil, where hyphæ usually are produced in place of the sporidia. The effect of very high temperatures is evident in the type of germ tubes. At the lowest temperatures the tubes appear to be normal, whereas, at temperatures around 29° and 30° C., the sporidia sometimes seem not to be completely formed and the tubes tend to be thin and transparent. At temperatures around 32° the tubes are barely visible and never grow to the usual length.

THE INFLUENCE OF MOISTURE ON SPORE GERMINATION

The object of this phase of the work was to determine the effect of the moisture content of the soil on the spores of *Ustilago avenæ*. Various methods, whereby spores could be placed in the soil under conditions similar to those obtaining in nature and recovered for examination under the microscope, were considered. The following technique finally was adopted. About 30 cc. of 2.5 per cent water agar of known acidity (P_H6.4 or +0.4 Fuller's scale) was poured into open petri dishes and dried thoroughly at a temperature not above 60° C. From the dried agar, smooth pieces 1 cm. square were cut. To distribute the spores evenly over the pieces of agar, a surface of paper was covered as evenly as possible with the spores. The pieces of agar were then laid one by one upon this surface and pressed gently to bring the whole of the surface in contact with the spores. Pieces of filter paper then were clipped above and below the agar with a fine wire. The spores thus prepared were sown under the same conditions as the oat seeds, 1 inch below the surface of the soil.

The soil moistures were made up on the basis of the moisture-holding capacity, as in the experiments of Bartholomew and Jones (*2*), and the acidity determined by Truog's (*21*) method. To insure uniform moisture distribution in the soil, either water was added to the soil 24 hours previous to sowing, or moistened soil was mixed before using, except that the 80 per cent was made originally to 60 per cent and after seeding was brought to 80 per cent. Uniform temperatures were maintained by incubators. The germination of the spores on the agar layer could be counted under the microscope after the removal of the filter paper.

At this point the question will arise as to how closely the conditions to which the spores were subjected in these experiments approximate the actual conditions to which the spores are exposed when naturally distributed in the soil. The availability of water to the spores is probably not the same when the spores are in contact with the agar as when they are in contact with the soil. In order to determine whether the amount of water absorbed by the agar varies directly with the moisture content

of the soil the following experiment was performed. Layers of oven-dried agar were weighed, clipped between filter papers, buried for 24 hours in cans of soil, the moisture content of which was made up to definite percentages, and then removed and weighed. The quantity of distilled water absorbed by 1 gram of oven-dried agar in 24 hours at 22° to 23° C. was found to be 11.8 grams. This maximum quantity of water absorbed under these conditions may be considered the water-holding capacity of agar. In the same time and at the same temperature similar agar placed in samples of a soil containing, respectively, 80 per cent, 50 per cent, and 20 per cent of its moisture-holding capacity, absorbed, respectively, 55 per cent, 30 per cent, and 10 per cent of its water-holding capacity. This shows that agar absorbs water approximately in proportion to the amount in the soil and may be interpreted as indicating that the amount of water available to the fungus fluctuates with the moisture content of the soil. It would seem that, as far as the other factors in the soil are concerned, the conditions to which the spores were subjected very closely approximated those occurring in nature, and it will so be assumed in the remainder of this paper.

In the first moisture experiments, the soil temperature was maintained between 20° and 24° C., and germination was recorded between 24 and 27 hours after sowing. Table III gives a summary of the results of these experiments.

From Table III it will be seen that the production of sporidia, or the small hyphæ which replace the sporidia, seems to be associated with conditions favorable to good germination, and occurs less frequently at 80 per cent soil moisture.

TABLE III.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at 20° to 24° C. in 24 to 27 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
9.....	21	Threads, buds,[1] sporidia.	21	Threads.....	4	Short tubes, long tubes, buds.
11.....	19	Threads, sporidia.	19	.....do.....	0	
2.....	35	Threads, buds; sporidia rare.	28	Threads rare; buds abundant; very many short tubes.	.6	Tubes just visible; one long with thread.
2.....	16	Sporidia abundant; threads, buds, long tubes.	11	Long tubes, threads, buds.	5	Threads.
5.....	16	Very long tubes, threads; sporidia abundant.	10	Threads.....	5	Do.
5.....	18	Threads, sporidia very rare.	15	.....do.....	7	Few threads; long and short tubes; buds.
Average.	21	.....	17	.....	4	

[1] Threads are the infection hyphae which may replace sporidia. Buds, a very early state of infection hyphae or sporidia.

It was next desired to ascertain whether the same general relation of spore germination to varying soil moistures obtains at other temperatures. The low temperatures of 10° to 13° C., and the high temperatures of 30° to 33° were chosen for this study. The duration of the different experiments was determined by the length of time required for spore germination at those temperatures. Tables IV and V show an agreement with Table III, and the three tables make clear that at these different temperatures germination is highest at 30 per cent soil moisture, is usually less at 60, and falls off very markedly at 80.

TABLE IV.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at temperatures of 10° to 13° C. in 48 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
9.....	24	Threads, some short tubes, long tubes, buds.	26	Threads very long; tubes, buds.	8	Threads, short and long tubes.
2.....	9	Sporidia, threads.	3	Long tubes.....	2	Short tubes.
5.....	9	Sporidia rare.....	5	Threads, long and short tubes.	5	Threads.
Average.	14	.....	11	.....	5	

TABLE V.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at temperatures of 30° to 33° C. in 24 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
10.....	4	Short tubes, no sporidia, some tubes long, some buds.	3	No sporidia or threads, short tubes mostly just visible.	0.06	Tubes just visible.
2.....	12	Long tubes with few threads.	4	Tubes long and just visible.	.20	Do.
5.....	10	Long tubes, buds.	13	Threads, buds....	3.0	Threads, short and long tubes.
Average.	8	.....	7	.....	1.0	

These results raise the question of the reason for the decrease of spore germination at the higher moisture contents. An increase in water might bring about a change in other factors affecting germination. These factors may be markedly modified when the puddling point is reached. At 80 per cent soil moisture, puddling is easily brought about

by such mechanical agencies as jarring or rapid addition of water. It was observed that when such a point was reached in a few of the preliminary experiments, germination was very much lower than in the later experiments when puddling was avoided by very careful handling of the soil. It is obvious, then, that the condition of the soil containing 80 per cent of its moisture-holding capacity was not far removed from that of puddled soil. Among the several factors that may have been responsible for reduced germination in wet soil, that of absence of sufficient oxygen appears the most probable. For this reason experiments were carried on to determine whether the spores germinate in the absence of oxygen.

THE INFLUENCE OF OXYGEN ON SPORE GERMINATION

In the search for a method whereby oxygen could be excluded from a spore suspension which could be kept under observation, many devices were tried before a satisfactory technique was finally adopted. The apparatus used is shown in Figure 3. Oxygen-free air was forced through an Engelmann cell (fig. 3, C) where spores were suspended in beef broth (P_H 6.4), as in the germination experiments previously described. The beef broth had been freed from oxygen by heating at 100° C. and kept in an air-tight receptacle with a solution of pyrogallic acid. The oxygen was removed from the air in the air-tight bottle (fig. 3, B) by introducing a liter of alkaline pyrogallic acid and shaking it within the bottle for 20 minutes. Normal pressure was restored after shaking by siphoning boiled water from the reservoir (fig. 3, A) into the oxygen-free bottle. The tube from the reservoir to the bottle was then tightly clamped, the reservoir covered with a quarter of an inch of kerosene to exclude oxygen, and the latter raised to the position shown in the figure.

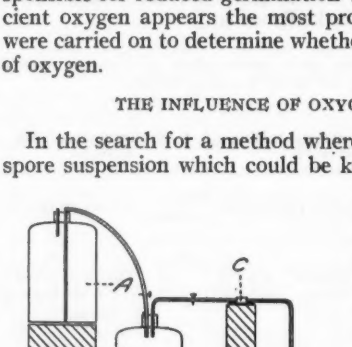


Fig. 3.—Apparatus for excluding oxygen from a spore suspension: A, reservoir of oxygen-free water; B, bottle containing pyrogallic acid; C, Engelmann cell; and D, bottle of water.

The Engelmann cell with its spore suspension was then attached to the outlet tube of the bottle, and a flow of water allowed to pass through the siphon into the bottle of oxygen-free air whose connection with the Engelmann cell was then opened. The oxygen-free air thus forced through the Engelmann cell was allowed to bubble through water in a small bottle placed at a much lower level (fig. 3, D).

By regulating the inflow of water from the reservoir, the oxygen-free air was forced rapidly through the cell for at least 40 minutes, and then more slowly for the remainder of the 24-hour period, except during the time when the air passing through the cell was collected for analysis in a Hempel pipette by the replacement of mercury. During each experiment four or five pipettes of gas were collected and the percentage of oxygen determined over phosphorus.[3] This analysis was made in order

[3] The writer is indebted to Dr. A. S. Loevenhart and the Department of Pharmacology, University of Wisconsin, for the use of the oxgen-analysis apparatus.

that the presence of oxygen entering the apparatus from any source might be detected.

With the apparatus in this final arrangement, three spore suspensions were thus subjected to an atmosphere free from oxygen as determined by the analysis of the samples of air passing over them. In no case did these spores germinate. Control mounts in Van Tieghem cells showed good germination. Before the apparatus was perfected and gas analysis made at the end of each germination, 15 trials had been made in which germination had occurred in only two instances when leaks in the apparatus, which admitted oxygen, were subsequently detected. The data confirm the results of the three final trials cited, and strengthen the evidence that *Ustilago avenae* spores will not germinate in the absence of oxygen. Some of these trials, however, have indicated that the spores will germinate in a low percentage of oxygen. In two experiments, germination of 5.9 per cent was found when the oxygen percentage was 5.85 and 1.95. The controls gave 28 and 35 per cent. In another trial in which germination was 27 per cent, and the control germinated 21 per cent, the gas analysis showed 0.5 per cent of oxygen.

DISCUSSION AND CONCLUSIONS

From the temperature experiments, it may be concluded that within the range from 9° to 28° C. there is no temperature effect upon the fungus which may prevent infection and establishment of the fungus within the host. According to Bartholomew and Jones (*2*) the range of temperature over which the highest percentages of smut occur is from 12° to 28°. This is nearly covered by the optimum range for germination of the fungus, 15° to 28°. Here also, according to Haberlandt (*8, p. 43*), is included the optimum for the most favorable and rapid germination of the host; namely, 25° C. At 9°, where the percentage of smut is either zero or very low, it takes about 10 days for the host to appear above ground and from four to five days for a good germination of the fungus and subsequent sporidial production. It would seem that the lack of smut was in no way due to an unfavorable influence of temperature upon the fungus alone. At 29° and below 6° C., however, the temperature seems to be unfavorable to the fungus. At the first and above, the germination is very much reduced and is abnormal, while sporidial production is rare. Here, it seems clear, a temperature effect may be the cause of the low percentage of smut. Below 6° it is possible that the host may develop before the germination of the fungus takes place, as the fungus requires about nine days to produce any considerable germination. These temperature conclusions are essentially in agreement with those of Ravn (*20*), Tubeuf (*22*), Eriksson (*7*), and Heald and Zundel (*11*).

As Bartholomew and Jones (*2*) found a low percentage of smut at the high soil moistures, and as at 80 per cent soil moisture there is a decidedly low fungal germination, with a slight falling off at 60 per cent as compared with 30 per cent, it seems safe to conclude that at the high content of soil moisture the decrease in spore germination may be a direct cause of the low percentage of smut. Analyzing this still further, we can perceive indications that this may be due to a factor involving oxygen supply. Were the oxygen completely eliminated at these high moisture contents, it could be stated conclusively that the absence of oxygen prevented the smut germination, for these experiments have demonstrated for this fungus the commonly assumed hypothesis that the spores

will not germinate in a liquid which is not exposed to oxygen. On the other hand, these experiments also indicate very strongly that with reduced oxygen supply germination still may take place. If it were true that in a soil of high moisture content some smut spores were in contact with oxygen and others were not, the reduction in germination would be clearly explained.

SUMMARY

(1) In order to determine the effect of temperature upon the germination of the spores of *Ustilago avenae*, spores were germinated in Van Tieghem cells or hollow-ground slides at a series of temperatures ranging from 4° to 35° C. in beef broth +10 Fuller's scale (P_H 6.1). The optimum for germination is arbitrarily defined as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours. The minimum temperature for germination was found to be between 4° and 5° C., the optimum from 15° to 28°, and the maximum between 31° and 34°.

(2) Under these conditions, the minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from 29° to 30° C.

(3) The relation of moisture to spore germination was studied by placing the spores on layers of agar between filter papers in soil containing 30, 60, and 80 per cent, respectively, of its moisture-holding capacity. Germination was found to be highest at 30 per cent, to fall off slightly at 60 per cent, and very markedly at 80 per cent.

(4) *Ustilago avenae* spores failed to germinate in a suspension exposed to an oxygen-free atmosphere.

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INFLUENCE OF TEMPERATURE ON THE SPORE GERMINATION OF USTILAGO ZEAE [1]

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Temperature has been regarded by investigators as important among the environmental conditions influencing the infection of corn by *Ustilago zeae* (Beckm.) Unger. Maire (*5*)[2] found that the spores of the corn smut fungus "germinate more quickly if the temperature is a little raised (20° to 25°)" and that the optimum temperature for sporidial and filamentous development is 20° to 25° C. Piemeisel (*7*), in considering the "phenomenon of infection and the optimum conditions for the propagation of the fungus," found the optimum temperature for the budding of sporidia to lie between 20° and 26° C., the maximum at about 40° and the thermal death point near 46°. He also found that incubation at 24° to 38° did not seem to influence the rate or amount of germination of the smut spores.

As the germination of the spores themselves would seem to be at least as significant as the budding of sporidia, and as the above investigators do not give detailed experiments on this stage of fungal activity, a study of the influence of temperature on the spore germination of *Ustilago zeae* was undertaken in order to throw additional light upon the relation of temperature to this early, significant stage in the life history of the fungus. The studies of which the results are presented in this paper have been carried on under the direction of Dr. L. R. Jones.

In searching for suitable media for spore germination it was attempted to duplicate natural soil conditions. Brefeld (*2*, *p.* 67–75) performed experiments which indicated that the germination of the *Ustilago zeae* spores takes place in the soil. The conception of soil, especially that containing manures, as a usual medium of germination of the spores also has been held even down to the present time. In attempting to find a soil medium like that obtaining in nature, the method of Thompson (*8*) was tested. When a tap-water suspension of spores was poured onto loam, no germination took place, whereas, when a suspension of spores in Pasteur's solution was introduced in the same way, germination followed. It seemed that the conditions for spore germination in such soil were not suitable, whereas they were satisfactory in the Pasteur liquid. In an attempt to find a soil on which the spores would germinate, seven modifications of loam and manure were employed, and the spores of *Tilletia tritici* (Bjerk.), Wint., were used as controls. On one pot which contained cow manure and loam, 1:2, there was considerable germination, and on another pot, one germinating spore was observed. Another test gave slight germination. In all these tests the bunt spores germinated abundantly, but, as a result of all the 27 attempts to germinate the spores of *Ustilago zeae* on soil, no satisfactory method was found.

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The investigations, the results of which are presented in this paper, were conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[2] Reference is by number (italic) to "Literature cited," p. 596–597.

An artificial medium was used, therefore, in the study of the effect of temperature on spore germination. Arthur and Stuart (*1*), Brefeld (*2*), Clinton (*3*), Hitchcock and Norton (*4*), Maire (*5*), and Norton (*6*) germinated the spores in many artificial media. Of these, Pasteur's solution was found most favorable for the following experiments.

The spores from a pustule formed in an anther were suspended in a tube of medium, mounted in Van Tieghem cells, and subjected to 13 different temperatures between 8° and 37° C. in thermo-regulated incubators. Duplicate mounts were placed at each temperature and the experiment performed 11 times. To determine the cardinal temperatures for germination, the percentage of germination was counted; the sporidial production, the number of cells in the promycelia, and the cell contents were observed; and the length and width of the germ tubes measured.

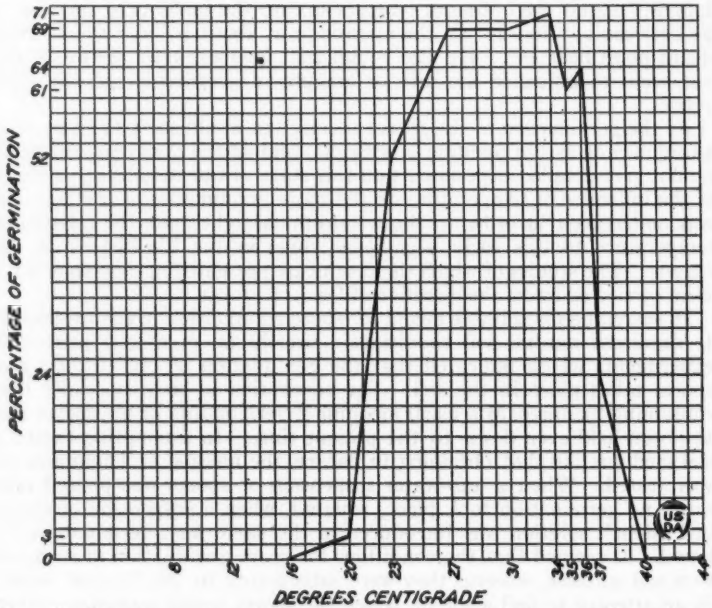

FIG. 1.—Graph showing percentages of germination of chlamydospores of *Ustilago zeae* at different temperatures.

The exact minimum for spore germination of *Ustilago zeae* was not determined. Four experiments showed germination at an average temperature ranging from 7.9° to 8.4° C., the germ tubes appearing on the 12th, 15th, 16th, and 21st days, respectively. The temperature range of the individual tests was greater than desired. That, together with the daily removal of the mounts from the incubators, may have given sufficient opportunity for eventual germination. The optimum temperature for germination is arbitrarily defined as that at which there is the highest percentage of germination within 24 hours together with a production of such germ tubes as behave normally, especially with respect to sporidial production. The highest percentage of germination within 24 hours occurred between 26.5° and 34.7° C. as is shown in Table I and figure 1, which give the average results of all experiments. As

TABLE I.—*Results of experiments on the germination of Ustilago zeae spores, showing range of temperature, time required for sporidial production, length and contents of germ tubes, and percentage of germination at different periods after sowing.*

Number of experiments included.	Average of temperature ranges.	Sporidial production.		Germ tubes.		Germination on given days after sowing.								
		Number.	Time.	Length.	Contents.	¾	1	2	3	4	5	6	7	16
	C.		*Days.*	*μ.*		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
5	7.9–8.4	Present	15–21	43	Granular; some vacuolate									4
5	11.7–12.9	None to abundant	3–6	32	do		0	0	0.7	3	11	44	57	
5	15.3–16.9	Present to abundant	2	36	Granular; granular and vacuolate.		0	33	58					
5	19.0–20.9	Few to abundant	1–2	37	Granular; some vacuolate	0.004	3	69						
4	23.0–23.9	Present to abundant	¾	31	Granular and vacuolate	13	52	73						
5	26.5–28.1	Abundant	¾	33	do	54	69							
11	30.0–32.9	do	¾	45	Granular; granular and vacuolate.	52	69							
6	34.0–34.7	Mostly abundant	¾–1			39	71							
6	35.0–35.9	None to abundant	¾–1			41	61							
4	36.0–36.9	Few to abundant	1–6	59	Homogeneous	24	64							
7	37.0–37.8	None to many	¾–2	59	do	14	24							
9	39.7–40.8	None				0	0	0	0	0	0	0	0	0
3	44.7–44.9	do				0	0	0	0	0	0	0	0	0

determined by the number produced on first observation, the optimum temperature for sporidial production lies between 26.5° and 34.7° C. The maximum lies between 36° and 38°. Of 20 observations above 34° only 4 showed abundant production.

At the low temperatures, wherever germination took place sporidia also were produced. The number of cells in the basidia and the width of basidia were alike at the different temperatures and so could not be used as criteria in establishing the optimum temperatures. The lengths of basidia and condition of cellular contents, as shown in Table I, differed at 36.1° to 37.8° C. from those at other temperatures, indicating a deviation from normal at this high temperature. As below 26.5° the percentage of germination is decreased, and, as above 34.7° the sporidial production is decreased and germ tubes are abnormal, the optimum temperature for spore germination may be stated as between about 26° and 34° C. As Table I shows that the maximum temperature rests between 37.1° and 37.8° and as six experiments show no germination at temperatures above 38° after two weeks, the maximum temperature for germination apparently lies between 37° and 38° C.

Attention should be called to the fact that this optimum temperature for germination is much higher for *Ustilago zeae* than for other smuts which have been studied in this laboratory. For example, *Ustilago avenae* germinates best between 15° and 28° C., and, according to Volkart (9), *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn germinate best between 16° and 18° C.

SUMMARY AND CONCLUSIONS

(1) In 27 trials to germinate *Ustilago zeae* spores in soils containing different amounts of manure, there was considerable germination in only one, slight germination in another, and germination barely occurring in a third. The spores germinated readily in many artificial media, from among which Pasteur's solution was chosen for the experiments to determine the relation of temperature to spore germination.

(2) The optimum temperature for the germination of *Ustilago zeae* spores was found to lie between about 26° and 34° C., the maximum between 36° and 38°, and germination was observed to occur at the minimum temperature of 8°. The optimum for sporidial production lies also between about 26° and 34° C., the maximum between 36° and 38°, and sporidia are produced at the lowest temperatures at which germination was observed.

(3) The experiments showing that high temperature is most favorable for spore germination indicate that infection likewise is favored by hot weather whether the germination takes place in the soil or in water held upon the host plant.

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SPORES IN THE UPPER AIR[1]

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INTRODUCTION

As a part of the rust-epidemiology investigations which have been made since the spring of 1917 by the Office of Cereal Investigations of the United States Department of Agriculture in cooperation with several State experiment stations, attempts have been made to get as much information as possible on the dissemination of spores by air currents and to correlate the data with the spread of rust on host plants. The usual method in studying the distribution of spores of pathogenic fungi by wind has been to expose spore traps of various kinds near the surface of the earth. The possibility of spores being carried to higher altitudes by convection currents, whirlwinds, and other air movements has been recognized, and spore traps have been exposed on high buildings, on mountain tops, and other elevated points. However, it is desirable to know how many spores there are in suspension several thousand feet above the surface of the earth.

During the spring and summer of 1921 [3] spore traps [4] were exposed on airplanes [5] in the hope of obtaining more information on the dissemination of aeciospores and urediniospores of *Puccinia graminis* and other rust fungi. The general results seem to be worth recording, although the problem of rust epidemiology was not solved.

METHODS

Airplanes were used in preference to balloons, kites, or other similar devices, because long distances could be covered in a short time, spore traps could be exposed easily at different altitudes, and the direction of flight could be changed at will.

Ordinary microscope slides (3 inches by 1 inch), smeared lightly with vaseline on one side, were exposed in different ways. One method was

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[2] The authors wish to acknowledge the enthusiastic cooperation of the commanding officer at Kelly Field, San Antonio, Tex.; Maj. Ira A. Rader, air officer for the Seventh Corps Area of the United States Army, at Fort Crook, Nebr.; Mr. W. I. Votaw, manager of the Aerial Mail Service Station at Omaha, Nebr.; and Mr. W. A. Kidder, of the Curtiss Flying Field (private), St. Paul, Minn. The authors are under deep obligation to these men, and to the pilots working under their direction, for their generosity and courtesy.

[3] The manuscript containing the results obtained in 1921 was submitted for publication before the 1922 results were available. On account of the delay in publication it has seemed desirable to include a few of the most important results obtained in 1922, although details are not given.

[4] The mechanical spore trap was devised by G. D. George, of the Office of Cereal Investigations and the University of Minnesota.

[5] The Air Service of the United States Army furnished the planes and pilots for the flights. The following men assisted in the work for the U. S. Department of Agriculture: R. B. Lambert, E. H. Ostrom, J. J. Christensen, D. L. Bailey, Wallace Butler, and S. P. Harter.

for the operator, seated in the passenger cockpit of the plane, to expose the slide by hand for the desired length of time and then return it to the slide box in which it had been carried. Another method consisted in placing inside of a bottle a microscope slide attached to a wooden paddle, the upper part of which extended through a hole in the cork of the bottle and served as a handle. After the slide had been exposed it was again placed in the bottle. This obviated the necessity of opening the slide box in order to remove or insert a slide, but was rather cumbersome, as it was necessary to carry as many bottles as there were slides to be exposed.

A third method was to expose the slides in a mechanical spore trap. Several types of trap were used, but one devised by Mr. George was the most satisfactory. This was made with six compartments, each containing a slide. It was fastened to the wing struts of the plane and was provided with a wire control which was operated by the observer in the cockpit. One pull on the wire opened the first compartment and exposed slide No. 1; a second pull closed this compartment and terminated the exposure of the slide. The other compartments were opened and closed in the same manner. (Pl. 2.) By this method slides could be exposed, one at a time, for the desired length of time, at various altitudes, and at widely separated points.

Flights were made in southern Texas during the latter part of April and during May, at St. Paul on June 25, and between Omaha, Nebr., and Cheyenne, Wyo., in July, 1921. The exposed slides were sent immediately in tight boxes to St. Paul, where spore counts were made.

RESULTS

Some typical results of exposing slides are summarized in Tables I and II. It is evident that there are many fungous spores at altitudes as high as 11,000 feet above the surface of the earth. In examining the slides special attention was given to rust spores. However, a great many spores of many other kinds of fungi, as well as conidiophores, numerous pollen grains, glumes of grasses, and some small insects also were caught on the slides.

It was practically impossible to identify many of the spores definitely. It was certain, however, that there were many spores of Puccinia, Alternaria, Helminthosporium, Cladosporium, Cephalothecium, Ustilago, Tilletia, and Scolecotrichum. (Pl. 1.) Spores of Alternaria were by far the most numerous, and not infrequently they were found adhering in characteristic chains. Chains of Cladosporium spores also often were found on the slides. On some of the slides there were large clumps of smut spores, there being approximately 60 in one of the largest clumps. Urediniospores were caught more commonly than any of the other spore forms of rust fungi, although teliospores were not uncommon, and, in 1922, aeciospores also were caught. On one slide there was a spore of what appeared to be *Ustilago zeae* with a promycelium and about 20 sporidia still attached. It is possible that this spore may have germinated in the vaseline. This seems quite unlikely, however, as there was no indication whatever that any of the other spores had germinated, and it seemed more probable, therefore, that the spore with its promycelium and attached sporidia was carried up into the air after it had germinated.

TABLE I.—*Results of exposing slides on airplanes*

Slide No.	Location.	Date.	Altitude (above ground).	Length of exposure.	Spores and pollen grains on slides.						
					Stem rust, uredinio-spores.	Leaf rust,[1] uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	Total.
			Feet.	*Minutes.*							
1	San Antonio, Tex.	Apr. 27	1,000	[2] 1	....	5	1	....	....	28	34
2	.....do.	...do.	3,000	5	....	8	5	....	3	28	44
3	.....do.	...do.	3,000	5	....	13	....	....	8	47	68
4	.....do.	Apr. 28	4,000	[2] 1	1	29	....	....	6	29	65
5	.....do.	...do.	Control.	.....	....	....	....	....	....	....	0
6	Waco, Tex., to San Antonio, Tex.	Apr. 29	500 to 1,500	30	....	355	1	1	3	21	381
7	.....do.	...do.	1,500 to 3,000	30	....	335	3	1	23	38	400
8	.....do.	...do.	1,500 to 3,000	25	....	104	....	....	9	50	163
9	.....do.	...do.	1,500 to 3,000	25	....	70	....	....	8	144	222
10	.....do.	Apr. 28	10,000 to 12,000	15	....	1	....	....	....	....	1
11	.....do.	...do.	12,000 to 14,000	15	....	....	....	....	1	....	1
12	.....do.	...do.	14,000 to 15,000	10	....	....	....	....	....	....	0
13	.....do.	...do.	15,000 to 16,000	10	....	....	....	....	....	....	0
14	.....do.	...do.	16,000 to 16,500	10	....	2	....	....	....	....	2
15	San Antonio, Tex.	May 2	1,000	20	....	366	5	2	17	60	450
16	San Antonio, Tex., to Uvalde, Tex.	May 23	Control.	10	....	....	....	....	....	....	0
17	.....do.	...do.	1,000	2	3	23	15	2	5	....	48
18	.....do.	...do.	1,000 to 2,000	10	28	33	13	....	14	5	93
19	.....do.	...do.	1,000 to 2,000	30	80	86	36	1	15	....	218
20	.....do.	...do.	2,000 to 2,500	20	16	32	1	1	25	....	75
21	.....do.	...do.	8,000 to 9,000	5	4	36	10	1	9	....	60
22	.....do.	...do.	9,000 to 10,000	5	....	9	19	....	5	....	33
23	.....do.	...do.	10,000 to 11,000	5	....	4	3	....	12	1	20
24	.....do.	...do.	11,000 to 12,000	5	2	7	1	....	....	....	10
25	San Antonio, Tex., to Cuero, Tex.	...do.	Control.	10	....	....	....	....	....	....	0
26	.....do.	...do.	1,000 to 2,000	1	4	12	3	....	7	....	26
27	.....do.	...do.	3,000	10	40	30	12	....	15	15	112
28	.....do.	...do.	3,000	30	80	56	35	3	25	4	203
29	.....do.	...do.	3,000 to 6,000	10	34	35	3	....	10	....	82
30	Omaha, Nebr.	July 13	2,000	25	33	14	[3] ab	12	[4] n	15	....
31	Kearney, Nebr., to Gothenburg, Nebr.	...do.	6,000	25	4	3	34	3	14	12	70
32	North Platte, Nebr.	...do.	Ground.	Overnight.	2	13	10	2	7	6	40
33	North Platte, Nebr., to Ogalalla, Nebr.	July 14	2,500	25	19	36	23	2	9	22	111
34	Sidney, Nebr., to Pine Bluff, Wyo.	...do.	7,000	25	3	2	4	....	3	....	12
35	Pine Bluff, Wyo., to Cheyenne, Wyo.	...do.	8,000	30	1	....	5	....	....	....	6
36	Potter, Nebr., to Sunol, Nebr.	July 15	2,500	20	1	1	19	....	18	1	40
37	Gothenburg, Nebr., to Elm Creek, Nebr.	...do.	2,000	25	126	34	ab	38	ab	68	....
38	Elm Creek, Nebr., to Wood River, Nebr.	...do.	3,500	25	101	28	ab	92	ab	17	....
39	Valparaiso, Nebr., to Omaha, Nebr.	...do.	6,000	35	15	1	n	8	n	1	....
40	Cheyenne, Wyo.	July 19	6,500	5	32	17	n	4	n	12	....
41	Pine Bluff, Wyo.	...do.	8,000 to 9,500	15	48	25	n	4	n	18	....

[1] *Puccinia triticina* and *P. coronata*.
[2] Exposed by hand.
[3] ab=Abundant (over 200).
[4] n=Numerous (100 to 200).

TABLE I.—*Results of exposing slides on airplanes*—Continued

Slide No.	Location.	Date.	Altitude (above ground.)	Length of exposure.	Spores and pollen grains on slides.						
					Stem rust, uredinio-spores.	Leaf rust,[1] uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	Total.
			Feet.	*Minutes.*							
42	West of Pine Bluff, Wyo.	July 19	10, 000	5	44	20	[4]n	8	n	10	....
43	Grand Island, Nebr.	...do....	2, 000	10	[3]ab	n	ab	n	n	n	....
44	Big Springs, Nebr., to Sidney, Nebr.	July 22	3, 500	25	198	27	ab	....	ab	24	....
45	Sidney, Nebr., to Bushnell, Nebr.	...do....	5, 000	30	57	20	n	4	n	2	....
46	Lodgepole, Nebr., to Brule, Nebr.	July 23	4, 500 to 5, 000	30	7	11	n	2	n	....	....
47	Brule, Nebr., to North Platte, Nebr.	...do....	5, 000 to 7, 500	35	5	6	57	1	50	25	144
48	North Platte, Nebr.	...do....	Control.	.....	....	....	....	....	....	....	0
49	Curtiss Field, St. Paul, Minn.	June 25	500	2	2	2	....	....	....	....	4
50	.....do.....	...do....	1, 000	2	2	5	....	....	27	....	34
51	.....do.....	...do....	1, 500	2	3	8	....	....	1	....	12
52	.....do.....	...do....	2, 000	2	3	5	....	....	1	....	9

[1] *Puccinia triticina* and *P. coronata*. [3] ab=Abundant (over 200). [4] n=Numerous (100 to 200).

TABLE II.—*Results of exposing slides in a spore trap on the wing of an airplane and by hand from the cockpit for five minutes, at Fort Crook, Nebr., July 22, 1921*

Altitude.	Manner of exposure.	Spores and pollen grains on slides.					
		Stem rust, uredinio-spores.	Leaf rust, uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.
Feet.							
10, 500	In spore trap	0	13	21	0	5	2
10, 500	By hand	12	18	147	1	46	0
8, 000	In spore trap	15	2	90	5	65	7
8, 000	By hand	56	13	735	21	[1]ab	158
7, 000	In spore trap	14	1	65	5	48	1
7, 000	By hand	18	17	435	15	ab	36
6, 000	In spore trap	7	1	60	1	11	10
6, 000	By hand	6	15	ab	11	ab	50
3, 500	In spore trap	14	0	84	2	22	2
3, 500	By hand	14	0	ab	19	ab	11
2, 000	In spore trap	4	6	95	3	41	3
2, 000	By hand	32	35	ab	63	ab	17

[1] ab=Abundant (over 200).

TABLE III.—*Results of exposing slides, coated with vaseline or glycerine jelly, simultaneously by hand for 3 minutes on June 14, and vaseline-coated slides by hand for 10 minutes on July 5, from airplanes in the vicinity of a 40-acre tract containing large numbers of escaped common barberry bushes, near Waukegan, Ill.*

Slide No.	Date.	Altitude above ground.	Location.	Number of spores of *Puccinia graminis* on slides.			
				Aeciospores.		Urediniospores.	
				Vaseline.	Glycerine jelly.	Vaseline.	Glycerine jelly.
	1922.	*Feet.*					
1	June 14	100	Over bushes	4	6	0	0
3	...do....	1,000	.....do	5	15	2	1
4	...do....	2,000	.....do	6	6	1	2
5	...do....	5,000	.....do	1	7	0	0
6	...do....	7,000	.....do	3	0	0	2
7	...do....	10,000	.....do	0	4	0	0
8	...do....	12,000	.....do	0	1	0	0
10	...do....	2,000	10 miles away	4	5	1	2
11	...do....	2,000	15 miles away	2	2	0	1
12	...do....	2,000	25 miles away	1	0	0	0
13	July 5	100	Over bushes	14	.....	5	.....
14	...do....	100	.....do	8	.....	2	.....
15	...do....	1,000	.....do	7	.....	4	.....
16	...do....	1,000	.....do	10	.....	0	.....
17	...do....	2,000	.....do	11	.....	4	.....
18	...do....	2,000	.....do	9	.....	3	.....
19	...do....	6,000	.....do	4	.....	3	.....
20	...do....	6,000	.....do	7	.....	3	.....
21	...do....	2,000	Over Fort Sheridan	0	.....	2	.....
22	...do....	2,000	.....do	1	.....	1	.....

On a single one of the slides, exposed for five minutes at an altitude of 10,500 feet (about 2 miles), near Fort Crook, Nebr., 224 spores were caught. On a similar slide exposed for the same length of time at an altitude of 8,000 feet, 827 spores of known identity were caught, and in addition there were about 200 spores of unidentified forms, making the total about 1,000.

The highest altitude at which slides were exposed was 16,500 feet, or more than 3 miles above the surface of the earth. This slide was exposed between Waco and San Antonio, Tex., on April 28, 1921, and two urediniospores of what appeared to be *Puccinia triticina* were caught. It would not be safe, however, to conclude that spores normally occur at this height, as only a few flights were made at such extreme elevations. Spores are very numerous up to about 11,000 feet, but at higher altitudes they apparently are relatively scarce.

The number of spores caught per slide varied according to the method and place of exposure. Fewer spores were caught in the mechanical spore trap than on the slides which were exposed by hand. This probably was due to the fact that the spore trap was attached to the struts just above the upper surface of the lower wing, and it is quite probable that the air currents were deflected in such a manner as to miss the slides to some extent. By referring to Table II it will be seen that more spores were caught on slides which were exposed by hand from the cockpit than on those in the spore trap on the lower wing. In future work it probably would be well, therefore, to expose the slides on the fuselage or under the lower wing.

The real question, however, is not whether a great many spores are in the upper air, but whether they still are viable when they reach the surface of the earth. The difficulty of determining this conclusively is quite apparent. Germination tests were made, but some of them were inconclusive. The spores were embedded in vaseline, and when they were transferred to water they apparently did not imbibe much of it on account of the presence of the vaseline on the exospore. However, Alternaria spores which had been caught at 3,000, 4,000, 8,000, and 10,500 feet, respectively, germinated quite readily.

None of the urediniospores of *Puccinia graminis* germinated in 1921, but many of the tests were made too long after the spores were caught. In 1922, many more tests were made, and it was found that urediniospores germinated readily when caught at altitudes up to 7,000 feet. No tests were made on spores which had been caught at higher elevations. In one test, 64 per cent of the urediniospores germinated on a slide which had been exposed at 2,000 feet. The altitude at which the spores were caught apparently had little or no effect upon their viability. It seems quite likely, therefore, that urediniospores might be carried for long distances in the air and still retain their power to germinate. Germination tests were made of the spores on 24 different slides, and an average of 11.2 per cent of the urediniospores germinated. Considering the fact that the spores often were partially embedded in vaseline or glycerine jelly, a surprisingly large percentage germinated. A few æciospores which had been caught at an elevation of 1,000 feet also germinated. Many of the other kinds of spores also germinated very readily.

GENERAL DISCUSSION

The results of these preliminary experiments indicate that large numbers of spores and pollen grains are carried several thousand feet above the surface of the earth during the growing season. Probably they are carried long distances by the upper air currents, the direction and velocity of which are quite different from those near the surface. If the spores retain their viability, as some of them quite probably do, it is conceivable that a local epidemic might occur in one locality as a result of the blowing in of spores from an infection center in another distant locality. It would be particularly desirable to know more about the dissemination of the spores of those pathogens which often seem to be disseminated over wide areas in some almost miraculous manner, such for example, as *Phytophthora infestans* and various rust fungi.

Attempts were made in 1922 to get data regarding the spread of black stem rust. Slides were exposed on airplanes in Nebraska, Kansas, and Oklahoma, before stem rust had developed in those States but when it was already present in Texas. No spores were caught, however, until rust began to develop near the area in which flights were made. Flights were made from Fort Sill, Okla., toward Denison, Tex., when there was a considerable amount of rust near Denison but none in Oklahoma. It was quite clear that the number of spores in the air decreased rather rapidly as the distance from the rusted area increased. The distance to which spores may be carried undoubtedly depends on many factors, and no final conclusions can be drawn from a limited number of observations. Certainly aeciospores and urediniospores are carried up as high as 10,000 feet, and more, above the surface of the earth. Unless they are brought down by rain or some other agency,

they probably are blown long distances. Just how far they are blown and just how important they are in starting rust far from the place where they were produced can not be stated definitely. More observations will be made.

The airplane is a great aid in studying the distribution of spores of pathogenic fungi. It is likely to be very useful in epidemiology studies and it also may be useful in determining the value of establishing quarantine lines.

SUMMARY

(1) In the spring and summer of 1921 spore traps were exposed on airplanes at various altitudes and at several places in the Mississippi Valley.

(2) Many spores of several different genera of pathogenic fungi, conidiophores, pollen grains, glumes of grasses, and small insects were caught on the slides.

(3) Spores and pollen grains were relatively abundant at altitudes up to 11,000 feet. They were relatively scarce at higher altitudes, but two spores of what appeared to be *Puccinia triticina* were caught as high as 16,500 feet.

(4) Alternaria spores which were caught at altitudes of 10,500 feet and less were viable; urediniospores and aeciospores of *Puccinia graminis*, caught at elevations of 7,000 feet and 1,000 feet, respectively, also germinated.

(5) Airplanes probably will be useful in studying the dissemination of spores of many pathogenic fungi, and probably will aid in the solution of problems connected with the development of epidemics of plant diseases.

PLATE 1 [a]

Photomicrographs of spores caught on slides at various altitudes.

A.—Cluster of Ustilago chlamydospores (3,000 feet, near Wahoo, Nebr., July 16).

B.—Teliospore, *Tilletia tritici* spore, three rust urediniospores, and an Alternaria spore (1,500 feet, near Wahoo, Nebr., July 19).

C.—Helminthosporium spore (10,000 feet, Pine Bluff, Wyo., July 19).

D.—Chain of two Alternaria spores (1,000 feet, between North Platte and Gothenburg, Nebr., July 15).

E.—Alternaria spore, leaf rust urediniospore, and a stem rust urediniospore (2,000 feet, near Fort Crook, Nebr., July 22).

F.—Helminthosporium spore (1,500 feet, near Wahoo, Nebr., July 19).

G.—Urediniospore of stem rust, two Alternaria spores, and a Cladosporium spore (10,500 feet, near Fort Crook, Nebr., July 22).

H.—Cluster of rust urediniospores and one Alternaria spore (4,000 feet, between David City and Grand Island, Nebr., July 13).

I.—Alternaria spore and stem rust urediniospore (10,500 feet, near Fort Crook, Nebr., July 22).

J.—Two Alternaria spores and a Cladosporium spore (10,000 feet, near Pine Bluffs, Wyo., July 19).

K.—Scolecotrichum (?) spore (3,000 feet, near Wahoo, Nebr., July 16).

L.—Fusarium spore (10,500 feet, near Fort Crook, Nebr., July 22).

M.—Budding spores or sporidia, exact identity unknown (3,000 feet, between Meade and David City, Nebr., July 13).

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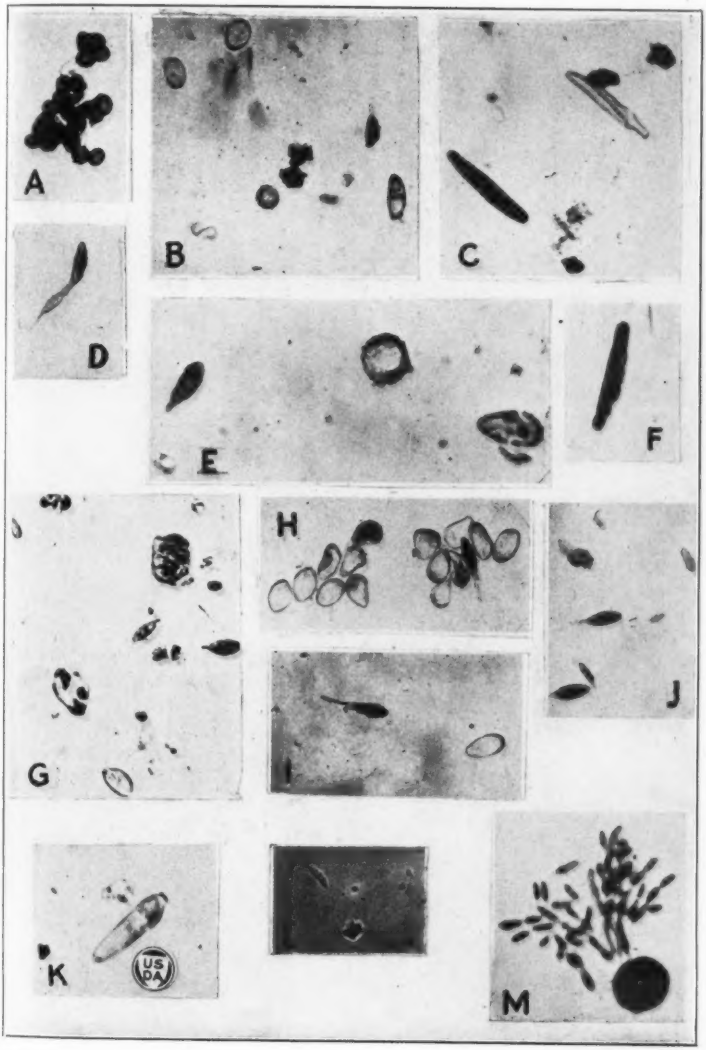
[a] Taken with the following combinations: A, K, and M, with Zeiss microscope No. 47303, 18 oc. and 8 mm. objective; E, with Leitz microscope No. 161036, 7.5 oc. and 6 mm. objective; remaining figures with Zeiss microscope No. 47303, 10 oc. and 8 mm. objective.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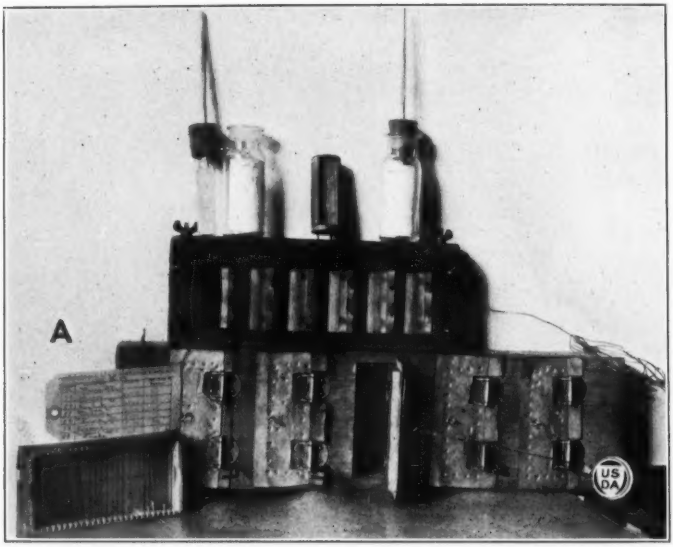
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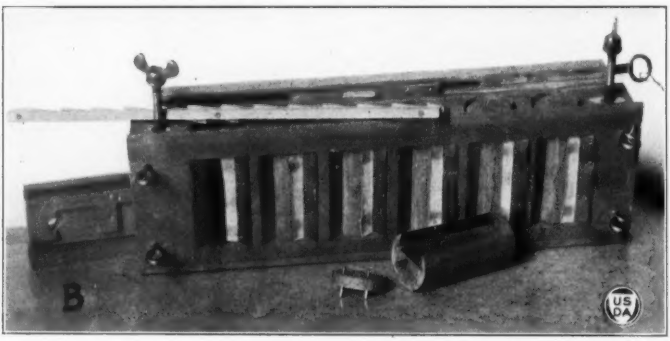
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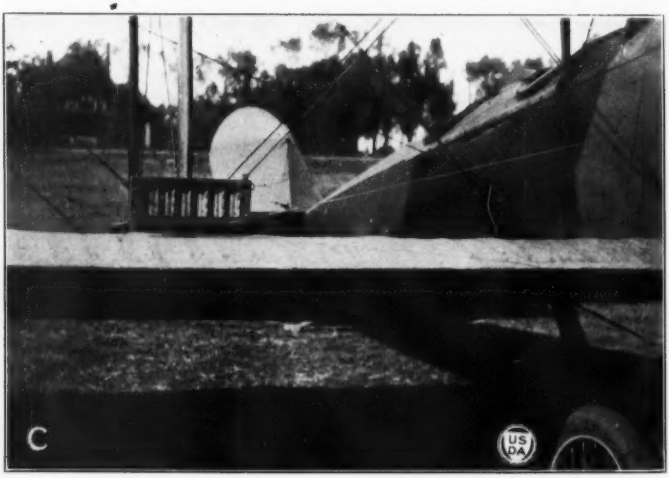
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PLATE 2

A.—Types of spore traps used.
B.—Construction of George spore trap.
C.—View of George spore trap attached to plane.

STUDIES ON THE LIFE HISTORY OF STRIPE RUST, PUCCINIA GLUMARUM (SCHM.) ERIKSS. & HENN.[1]

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INTRODUCTION

Extensive study has been made in European countries on the seasonal occurrence of the so-called yellow rust, caused by *Puccinia glumarum* (Schm.) Erikss. & Henn. It has been held by a few investigators in Europe that the disease may be transmitted from year to year by means of infected grain and grass seeds. Very little research has been carried on to determine if this is possible. The possibility of transmission of this rust through infected seed has an especial significance in the United States, due to its limited geographic distribution. It has been found that stripe rust in the United States is limited in its distribution to the States of the Pacific Coast and Rocky Mountain regions. If the disease can be carried on the seed, grain shipped from the Western States into the Mississippi Valley might become a means of spreading the rust into the Spring Wheat Belt.

It has been demonstrated by various workers that this rust can overwinter in the uredinial stage in central and northern Europe. It seemed important to know if this was true in the United States. Accordingly an investigation was undertaken, in cooperation with the Department of Plant Pathology of the Oregon Agricultural Experiment Station, in 1917, to work out the life history of this fungus under western conditions. The investigation was transferred, in 1920, to Moscow, Idaho, in cooperation with the Department of Plant Pathology of the Idaho Agricultural Experiment Station. The conclusions herein reported are the results of these investigations.

LIFE HISTORY STUDIES

OVERWINTERING UREDINIA

The aecial stage of this rust is unknown. Eriksson and Henning (*3*) were unable to find an aecial host for the rust, although they carried on rather extensive cultural experiments. It has not been possible so far to work upon this phase of the problem in connection with the present investigations. There have been no indications noted in the field which would suggest that there is an aecial host for the rust in this country.

It has been generally concluded by various authors in other countries that *Puccinia glumarum* is able to overwinter in the uredinial stage. Biffen (*1*)[3] states:

> The uredospore stage seems sufficient to enable the fungus to tide itself over the winter, for it is possible to find pustules of rust on the foliage of selfsown wheat or some-

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[3] Reference is made by number (italic) to "Literature cited," p. 620.

times on the ordinary autumn-sown crop even in the depths of winter. The twisted leaves lying on the soil form a series of sheltered moist chambers on the inner surface of which the rust pustules are occasionally present in great numbers. These may develop with great rapidity in the early spring and at times as early as March the whole of the plant's foliage may be yellow with the rust. The winter's cold does not appear to injure these spores for they germinate readily when brought into the laboratory.

Hecke (*4*) concludes that *P. glumarum* can overwinter in Austria. He has observed the disease breaking out in the spring upon the old spots on the leaves which had been infected in the preceding autumn. In about two weeks this would be followed by a second generation appearing on the new growth of the plants. He concludes that the conditions necessary for an epidemic of yellow rust in any given year are a sufficient number of overwintering plants carrying mycelia and meteorological conditions favorable for its development in the spring.

Eriksson and Henning (*3*) conclude that *P. glumarum* may overwinter as mycelium in grain seedlings, and that this method is especially significant in years when there is a continuous covering of snow on the ground throughout the winter. They were doubtful if this means of overwintering is sufficient to carry the rust over from year to year. Henning (*5*) in a recent paper has reviewed the literature upon overwintering of this rust in Europe. He finds that the reports from various countries in central and northern Europe show that this rust can overwinter, at least when the host plants are protected, in the shape of uredinial mycelium. Such overwintering has been observed in Sweden by Eriksson, Henning, and Klebahn; in Denmark by Rostrup; in Austria by Hecke; in Bohemia by Baudys; in Russia by Treboux; and in Germany by Kirchner.

According to the author's observation there does not appear to be any doubt that the uredinial stage of stripe rust in either its urediniospore or mycelial condition can survive the winter in the mild climate of the coast region of the Pacific Northwest. When the winters are mild, it is possible to find fresh uredinia developing in any month from September to July in this region.

In the autumn of 1917 numerous varieties of wheat were sown at Corvallis, Oreg., in order to test them for resistance to stripe rust. A guard row of Chul wheat, which is very susceptible to stripe rust, was sown around the entire nursery in which these varieties were being grown. This entire border row was inoculated on November 21 and December 3 with urediniospores of *P. glumarum*. Infection spread continuously throughout the winter until by January 30 infection was quite general on all susceptible varieties of wheat adjacent to the inoculated plants. By March 1, all the susceptible varieties were heavily infected.

During the winter of 1917–18 a number of pots of the club wheat known as Dale (Dale Gloria), which is a susceptible variety, were grown in the greenhouse and inoculated with urediniospores of stripe rust until they were heavily infected. They were then placed out of doors in an exposed place for five weeks of the coldest weather during the winter. Snow fell three times during this period, and the plants, which were 6 to 8 inches tall, were killed down to half their length by the frost. In six days after they were again taken into the greenhouse they were covered with urediniospores on the portions of the leaves which had not been killed.

Observations also show that the fungus can overwinter readily on various wild grass hosts. In the fall of 1917 and also in 1918, observa-

tion stations were established near Corvallis, Oreg., with a number of wild grasses which were naturally infected with stripe rust. Plants of *Hordeum nodosum* L., *Bromus marginatus* Nees, and *Elymus glaucus* Buckl. were chosen for these observations. At no time during either winter was it impossible to collect viable urediniospores at any of these observation points. Viable urediniospores were collected upon *Elymus glaucus* in 1917–18 in every month from September, 1917, to July, 1918, inclusive.

There is some evidence also which indicates that the fungus may overwinter in the uredinial stage in the intermountain regions, between the Cascade and Rocky Mountain Ranges. Willis (*11*) reports that the rust may overwinter at Moscow, Idaho, on *Hordeum jubatum*. The author also obtained evidence from observations made in the spring of 1920, and again in 1921, that the uredinial stage of the fungus may survive the winter in northern Idaho. Plants of *Hordeum jubatum* and *Bromus marginatus*, which were heavily infected in the fall, developed fresh uredinia in both 1920 and 1921, soon after the snow melted in the spring. In the fall of 1921, various infected hosts in the grass nursery at Moscow, Idaho, and also other hosts located at various places near Moscow, were marked and were examined as soon as the snow melted in the spring. Snow covered these plants continuously from the last week in December until the middle of March. On April 3, 1922, several uredinial sori were found on an old leaf of a seedling of *Hordeum nodosum* growing beside a mature plant of the same species which was heavily infected in the fall. None of these sori had broken through the epidermis of the leaf. The spores were perfectly normal although immature. The appearance of the leaf seemed to indicate that infection had taken place in the fall and after remaining dormant through the winter had started to develop thus early in the spring. By April 15 a number of seedlings of this grass were found infected and producing urediniospores in abundance.

OVERSUMMERING UREDINIA

Observations made during the last five years indicate that along the Pacific coast of Washington and Oregon it is much more difficult for the rust to survive the dry summer months of July and August than to pass through the winter. The summers of 1917 and 1918 were exceedingly dry in the Willamette Valley of Oregon where observations were made during those two years. In 1917 there was no rainfall from June 8 until September 10, and in 1918 from June 5 until September 12. Needless to say, practically all of the wild grasses were completely dried up and very little rust infection of any kind was to be found by September.

Observations were made at Corvallis, Oreg., throughout the summer of 1917 upon *Elymus glaucus*, *Bromus marginatus*, and *Hordeum nodosum*, all of which hosts were heavily infected with stripe rust during the month of May. The *Elymus glaucus* plants were located on the banks of a small creek near the college farm. Urediniospores were produced in abundance upon these plants during May and the first half of June. Infection became gradually less until about July 20, after which no new urediniospores were produced until after the fall rains began. During this period, from July 20 to September 8, the *Elymus glaucus* plants made very little new growth, but a few leaves retained their green color. The old lesions or stripes showed plainly upon these half-dried leaves, but

no new spores were produced thereon. Soon after the fall rains began, however, uredinia began to appear at the ends of the old stripes where the mycelium had survived. The rust developed and spread rapidly until by October 15 an abundance of uredinial infection could be found upon the plants of this species in this locality. This same method of surviving the dry summer months was noted in the same place during the summer of 1918 (Pl. I), and upon *Hordeum nodosum*, *Bromus marginatus*, *Elymus glaucus*, *Elymus canadensis* L., and *Hordeum jubatum* in the rust nursery.

Waterhouse (*10*) has reported that in the hot dry summers of certain parts of Australia, *Puccinia triticina* Erikss. survives the dry period on volunteer wheat.

It seems evident, then, that one of the determining factors for an epidemic of stripe rust in any given year must be the weather conditions during the summer and fall of the previous year. If the precipitation during these months is insufficient to keep the grass hosts of *P. glumarum* and volunteer wheat more or less green in order to tide the fungus over this rather critical period, very little infection material will be present to start an epidemic upon winter wheat. It seems to the writer that the months from July to November in each year may well be considered the critical period for the fungus in regions where the winters are more or less mild. The meteorological conditions in the spring would need to be such as to favor the spread of the fungus which had survived the winter months. However, if fall-sown wheat becomes well infected in the fall, the early spring conditions are usually more or less favorable for subsequent spread. This, of course, would apply only to winter wheat. The weather conditions in the spring would undoubtedly be the determining factor in the infection of spring wheat.

RESISTANCE OF UREDINIOSPORES TO DRYING

It has been shown above that uredinial mycelium of stripe rust may survive the dry summer months in the tissues of the host. It seemed also important to learn the length of time in which urediniospores might remain viable under various conditions. Eriksson and Henning (*3*) found that the urediniospores of this rust were difficult to germinate unless they had been chilled to the freezing point or lower. The writer has had no difficulty in securing good germination of fresh urediniospores at any time. A number of trials were made to ascertain how long urediniospores would remain viable when kept at room temperature in herbarium packets. Table I gives the results of several of these trials.

TABLE I.—*Percentage of initial and final germination of urediniospores of stripe rust, collected from different hosts, kept in paper packets at room temperature*

Host.	Date collected.	Initial germination.	Date of final count.	Final germination.	Total storage.
		Per cent.		*Per cent.*	*Days.*
Wheat	Dec. 1, 1917	80	Jan. 12, 1918	1	43
Do	Nov. 27, 1917	90	Jan. 19, 1918	Trace.	56
Agropyron dasystachyum	Aug. 28, 1916	95	Oct. 24, 1916	1	58
Elymus condensatus	Sept. 5, 1916	85	.....do.....	Trace.	49

An experiment also was conducted to compare the relative resistance to desiccation of urediniospores of several rusts. *Puccinia graminis avenae* Erikss. & Henn. on oats, *Puccinia triticina* Erikss. on wheat, *Puccinia holcini* Erikss. on *Holcus lanatus* L. and *Puccinia glumarum tritici* Erikss. and Henn. on wheat, were chosen. Leaves of the several hosts, heavily infected with the uredinial stage of the rusts, were collected and several portions of the infected leaves were placed in each of a number of small glass vials which were then corked. Other infected leaves of each host were placed in three series of small paper herbarium packets.

The three series of specimens in the paper packets were exposed to different sets of conditions. The first series was placed on a shelf in the open laboratory; the second, out of doors on a window ledge on the north side of the laboratory, and the third on a shelf in a culture room. The temperature and moisture conditions in this culture room were a little more constant, and both temperature and relative humidity were a little higher, than in the open laboratory.

The glass vials containing infected leaves were divided into four groups. The first was placed on a shelf in the open laboratory, the second out of doors on a window ledge, the third in the culture room mentioned above, and the fourth in a small desiccator, the bottom of which was filled with water. This desiccator was kept in the culture room with the other collections. The vials in the desiccator were not corked. Table II gives the results of both these experiments.

TABLE II.—*Resistance of urediniospores of various rusts to desiccation when stored in paper packets and in glass vials, under different sets of conditions*

Kind of rust.	Where exposed.	How kept.	Date collected.	Date of final count.	Final germination.	Storage period.
					Per cent.	*Days.*
Puccinia graminis avenae.	Laboratory	Packets	Nov. 13, 1918	Feb. 4, 1919	0	82
Do	Window ledge	do	do	do	12	82
Do	Culture room	do	do	do	0	82
Do	Laboratory	Vials	do	do	0	82
Do	Window ledge	do	do	do	Trace.	82
Do	Culture room	do	do	do	0	82
Do	Desiccator	do	do	do	0	82
Puccinia glumarum tritici.	Laboratory	Packets	do	do	[1] 0	82
Do	Window ledge	do	do	do	[1] 0	82
Do	Culture room	do	do	do	[1] 0	82
Do	Laboratory	Vials	do	do	[1] 0	82
Do	Window ledge	do	do	do	[1] 0	82
Do	Culture room	do	do	do	[1] 0	82
Do	Desiccator	do	Dec. 3, 1918	do	Trace.	63
Puccinia triticina	Laboratory	Packets	Nov. 14, 1918	do	0	81
Do	Window ledge	do	do	do	5	81
Do	Culture room	do	do	do	0	81
Do	Laboratory	Vials	do	do	3	81
Do	Window ledge	do	do	do	0	81
Do	Culture room	do	do	do	0	81
Do	Desiccator	do	do	do	0	81
Puccinia holcini	Laboratory	Packets	do	do	3	81
Do	Window ledge	do	do	do	5	81
Do	Culture room	do	do	do	Trace.	81
Do	Laboratory	Vials	do	do	Trace.	81
Do	Window ledge	do	do	do	Trace.	81
Do	Culture room	do	do	do	Trace.	81

[1] Trace of germination in 20 days

On January 8, 1919, urediniospores of *P. glumarum tritici* were taken from wheat leaves and placed on open slides and left under a bell jar in a culture room in the laboratory. The bell jar was raised slightly in

order to allow circulation of air. The spores were tested at the beginning and at intervals until no germination occurred. The germination at the beginning was found to be 76 per cent, at the end of 3 days it was 65 per cent, in 5 days 50 per cent, in 10 days 35 per cent, in 13 days 15 per cent, in 16 days 10 per cent, in 20 days 1 per cent, in 23 days only a trace, while in 25 days no more germination could be secured.

No extensive work has been done to ascertain how long urediniospores of *P. glumarum tritici* will remain viable in the field during the winter months. It is possible, as indicated above, to find viable urediniospores at almost any time during an open winter on the Pacific slope. It has not yet been possible, however, to learn if this is the case in the intermountain regions, where the winters are much more severe. Mr. A. F. Thiel states in a personal letter that he found, in connection with his studies of overwintering of stem rust in Montana, that urediniospores of *P. glumarum* were less resistant to the weather conditions than those of *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis* or *P. triticina*.

TIME OF NATURAL INFECTION

Stripe rust is very commonly found upon young seedlings and much has been made of this fact by the advocates of the theory of seed transmission of the disease. Experiments were arranged during the winter of 1917–18 to ascertain how early in its development a seedling might become infected. Seedling plants of Chul wheat were inoculated at various stages in their development, beginning as soon as the plumule emerged and continuing with different plants at intervals until the first leaf was fully expanded. No infection was secured in any case where the inoculation was made before the primary leaf had begun to expand. Twenty seedlings grown from seeds germinated in Petri dishes lined with filter paper were used in each case. When the primary leaf was well expanded, these were transferred to soil in pots.

The details of the experiment are as follows:

No. 1.—Urediniospores placed upon the plumule just as it began to appear. No infection resulted.

No. 2.—When the plumule was one-half inch long, no infection resulted.

No. 3.—When the plumule was 1 inch long, no infection.

No. 4.—When the primary leaf was just beginning to unfold from the sheath, but not well opened, 10 per cent infection.

No. 5.—When the primary leaf was well opened, 100 per cent infection.

Abundant infection has been noted upon the primary leaf of seedlings of *Bromus marginatus*, *Hordeum nodosum*, *H. murinum*, *H. jubatum* and *Sitanion jubatum* which came up in the rust nursery at Corvallis, Oreg., around old infected plants of the same species. (Pl. II, A.)

Uredinia appeared upon young seedlings of Chul wheat 21 days after it was sown on June 10, 1917, alongside of some winter wheat which was heavily infected with stripe rust. (Pl. II, B.) Some of the same lot of seed was sown some distance from any known infection. No stripe rust appeared on the plants from this seed until two weeks after it had appeared upon the seedlings mentioned above. This indicates that infection may occur under field conditions as soon as the primary leaf unfolds. It is very evident from observations made during the winter and early spring, that seedlings infected in the autumn may be a very

common means of carrying the rust over winter, at least on the Pacific Coast.

PERIOD OF INCUBATION

The length of the incubation period of the uredinial stage of various rusts does not appear to be the same. Parker (*8*) found that the incubation period of *Puccinia graminis avenae* Erikss. and Henn. and of *Puccinia lolii avenae* McAlpine, on oats, was about the same. He states, however, that generally, though not always, the uredinia of the stem rust appeared first. He took notes on the appearance of flecks on the seventh to the ninth day, and notes on the formation of uredinia in 12 days. He calls attention to the effect of temperature and light upon uredinia formation. Low temperatures were found to lengthen the period. Durrell and Parker (*2*) state that the incubation period for crown rust upon seedlings of a susceptible variety of oats averaged 7.8 days. On older plants of the same variety the incubation period averaged 9.5 days. Biffen (*1*) found that the incubation period of uredinia of *P. glumarum* on wheat was about 10 days. Marryat (7) states that pale yellow areas appear 6 days after inoculation of susceptible varieties of wheat with urediniospores of *P. glumarum*. Pustules begin to appear on the eleventh and are all open by the thirteenth day.

The author's observations in the greenhouse in connection with inoculation with urediniospores of *P. glumarum tritici* on wheat indicate that with optimum conditions for infection, namely, a susceptible host, fairly high temperature after the first 48 hours, good light, and vigorously growing seedling plants, infection begins to appear on the eighth or ninth day and is evidenced by lighter-colored areas on the inoculated portions of the leaves. Spores appear in abundance on the twelfth or thirteenth day. As observed by Parker (*8*), low temperature and lack of sunlight may materially lengthen this period. It was observed in 1918 in connection with inoculations which were made in the field at Corvallis, Oreg., late in the fall and early winter, that the incubation period at that time of year might be as long as 30 days. It seems possible that uredinial hyphae, after penetrating the host and becoming established, may lie dormant for a much longer time under conditions unfavorable to the development of the host and then again continue growth and the production of spores when growth of the host is resumed.

HEAD AND KERNEL INFECTION

A number of writers have called attention to the fact that both uredinial and telial infection of a number of rusts may be found upon the caryopses of various grains and grasses. The author (*6*) has summarized the literature regarding the occurrence of this phenomena and has called attention to the fact that kernels of wheat infected with *P. graminis tritici* are rather commonly found in wheat from badly rusted plants. The percentage of rusted kernels was not large, however, in any sample examined.

The development of both uredinia and telia of *P. glumarum tritici* upon all parts of the head and kernel of certain varieties of wheat has been found to be very common during severe rust epidemics. In many cases of severe infection it is impossible to find a place on the entire plant from the surface of the ground to the tips of the awns that is not

covered with rust sori. Head infection, which usually indicates that the kernels are more or less infected, is common only in certain varieties of wheat. Chul (C. I. No. 2406), Dale (Dale Gloria, C. I. No. 4231), Talimka (C. I. No. 2495), Baart (Early Baart, C. I. No. 1697), Little Club (C. I. No. 4219), Jones Fife (C. I. No. 3452), and a number of others, are very commonly infected in the head even when the rest of the plant may not be heavily infected. Many times, the inside of the glumes and the kernels of plants of the above-named varieties may be heavily infected without the development of open sori on the outer surface of the glumes. Except in cases of very severe infection, only a few of the spikelets on each head are infected.

If infection develops on the heads soon after they emerge from the boot, sterility of a large number of spikelets may result. (Pl. III, A.) The kernels which develop in such heads usually are very badly shriveled. (Pl. III, B, and Pl. IV, A.) It is easily possible to pick out infected heads even though there be no open sori on the surface of the glumes and awns. Such heads are much lighter in color, being a yellowish green, while the rest of the plant is a normal green. Plate IV, A, shows a number of detached glumes from heads of Chul wheat collected at threshing time. The inside of these glumes shows an abundance of stripe-rust telia.

Kernel infection by stripe rust is much more common in certain varieties of wheat than the author (*6*) found to be the case in wheat infected with stem rust. As high as 60 per cent of the kernels of certain varieties were found to be infected when grown in a rust nursery at Corvallis, Oreg., where a heavy infection of stripe rust was secured by artificial inoculation. Over 35 per cent of infected kernels were found in several samples of wheat grown under field conditions. It seems to the author that the amount of head infection, and consequently the amount of kernel infection, depends largely upon the climatic conditions at heading time, as well as upon the variety of wheat.

EFFECT OF KERNEL INFECTION UPON GERMINATION

The author has reported (*6*) that kernel infection by *P. graminis tritici* does not affect the germinating power of wheat to any appreciable extent. This has not been found true of wheat kernels infected with *P. glumarum tritici*. Numerous germination tests were made with several varieties of wheat which were badly infected with this rust. Parallel series of unrusted kernels from the same samples of seed also were germinated at the same time. The germination of the rusted seed averaged about 50 per cent of that of the unrusted seed. In some cases the germination was less than 25 per cent of that of the unrusted seed. By reference to Plate IV, B, it will be noted that infected kernels are very much shrunken. In many cases a cross section of an infected kernel revealed dozens of sori under the pericarp over practically the entire surface of the kernel. In the case of wheat kernels infected with *P. graminis tritici* the sori usually were confined to the hilar region and were present only in limited numbers.

SEED TRANSMISSION OF STRIPE-RUST INFECTION

HISTORICAL DISCUSSION

The author (*6*) has reviewed the principal literature upon the subject of rust transmission by seed grain and discussed the three theories put forth by various writers to explain the means by which such transmission may take place. These are the mycoplasm theory, the dormant mycelium theory, and the seed-borne-spore theory. The results of experiments carried on by various workers along this line have also been tabulated in the author's previous work (*6*) on this question. This review will not be repeated here. In this paper carefully controlled field and greenhouse experiments with wheat infected with *P. graminis tritici* were also conducted. The results of these studies led to the conclusion that stem rust is not transmitted from one wheat crop to the next by means of infected seed grain. Waterhouse (*9*), more recently, has carried on similar experiments with wheat kernels infected with stem rust which also gave negative results.

Certain European investigators have secured positive results in experiments carried on to determine if *P. glumarum* can be carried from year to year on seed grain. Because of the economic importance of this phase of the question in the United States in connection with the spread of the rust to regions where it is now unknown, experiments were undertaken with stripe rust both in the field and under carefully controlled greenhouse conditions.

EXPERIMENTAL DATA

A number of field observations have been made which at first seem to indicate very strongly that *P. glumarum* may be transmitted by means of seed. In the summer of 1917, among the increase plots of the Farm Crops Department at the Oregon Agricultural College, a plot of Jones (Winter) Fife was found to be heavily infected with stripe rust while none of the plots around it was infected to any extent. It was learned upon inquiry that the seed from which this plot was sown came from McMinnville, Oreg., and it was also learned that seed from the same lot sown at McMinnville produced plants that were not rusted. Upon close examination in the vicinity of the infected plots, numerous volunteer plants of Chul wheat were found which were heavily rusted. A plot of Chul wheat which was grown near this field the year before was known to have been infected with stripe rust. It seemed reasonable to suppose that the rust wintered over on these volunteer plants and infected the Jones Fife. No other very susceptible varieties were grown near this section of the field.

Another case in point which is not so easily explained was noted by Dr. A. G. Johnson, of the Office of Cereal Investigations, United States Department of Agriculture. Doctor Johnson found stripe rust on barley varieties in the nursery on the Belle Fourche Experiment Farm, at Newell, S. Dak., in August, 1917. These barleys were quite uniformly infected, and no other stripe rust was noted at Newell. The writer visited Newell in 1918, but was able to locate no stripe rust on or near the experiment station grounds. Some of the same seed from which these infected varieties had been grown was secured from Mr. John H. Martin, then of the Belle Fourche Farm, and sown in September, 1918. The plants from this seed were grown in the greenhouse at

Corvallis, Oreg., until they were headed but no rust appeared upon them. No rust infection could be found on this seed. Neither has the author been able at any time to locate any barley seed infected with *P. glumarum*. The work which is herein reported and the conclusions drawn relate to wheat only.

The investigations were along two lines: (1) Greenhouse experiments in which large numbers of rusted wheat kernels were sown under isolated conditions and the resulting plants watched for infection; (2) field experiments in which rusted wheat kernels were sown in the field and the resulting plants kept under observation to learn if infection occurred upon them sooner than upon plants grown from clean seed.

FIELD EXPERIMENTS

Rusted wheat seed has been sown for two years in comparison with clean seed and the resulting plants studied every few days for stripe-rust infection. The details and results of these experiments are given in tabular form, for each year separately.

In the autumn of 1917, rusted seed and chaff from rusted heads of several varieties of wheat were saved and sown in an isolated place on the college farm, near Corvallis, Oreg. The details of the experiment and the results secured are given in Table III.

These plots were examined carefully at frequent intervals in the fall and no stripe rust could be found on any of them at any time. In the spring the rust was slow to develop and did not spread rapidly, due to the extremely dry weather.

In 1919, a similar experiment was conducted under the same conditions. The details of the experiment and the results secured are given in Table IV.

As an additional precaution in securing seed which was known to be as free as possible from rust infection, all clean seed used was 1 year old.

The infection which appeared in the fall may have originated from some infected *Elymus glaucus* plants which were later found not far from these plots.

TABLE III.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1918, from rusted and unrusted seed sown on Oct. 10, 1917*

No.	Variety.	Condition of seed.	Number of rod rows.	Date rust first appeared.
1	Chul	Badly rusted seed and chaff	28	June 25, 1918
2	.....do	Portions of infected heads	3	June 20, 1918
3	.....do	Treated with modified hot-water treatment.	8	Do.
4	Talimka	Clean seed	2	June 22, 1918
5	Tigharia	Rusted kernels and chaff	3	June 25, 1918
6	Hansia Brooch	Rusted kernels	3	June 15, 1918
7	Popatia Nadiad	.....do	2	May 31, 1918
8	Jones Winter Fife	Clean seed	5	June 10, 1918
9	Chul	.....do	5	Do.

TABLE IV.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1919, from rusted and unrusted seed sown on Oct. 20, 1918*

No.	Variety.	Condition of seed	Number of rod rows.	First rust appeared.
1	Salt Lake Club	Slight infection	10	None until spring.
2	Chul	Heavy infection	10	Do.
3	Dale (Dale Gloria)	Clean seed	10	Do.
4	Baart (Early Baart)	do	10	Do.
5	Talimka	do	10	Do.
6	Dale	Medium infection	10	Do.
7	Federation	Clean seed	10	Do.
8	Chul	Heavy infection	10	Jan. 29, 1919.
9	Baart	Slight infection	10	Dec. 15, 1918.
10	Hansia Brooch	Heavy infection	10	Feb. 11, 1919.
11	Popatia Nadiad	do	10	None until spring.

GREENHOUSE EXPERIMENTS

In addition to the field experiments given above, greenhouse experiments have been carried on under controlled conditions. In order to grow a large number of wheat plants from seed infected with stripe rust, a portion of one of the greenhouses at the Oregon Agricultural College was equipped in the following manner: A partition was built to isolate completely one corner of the greenhouse; double doors were arranged in such a manner that anyone entering the isolated room could pass into a vestibule and close the first door before the second one was opened; the vestibule between the two doors always was sprayed with water before entering. A system of forced circulation of washed air was installed, as shown in figure 1.

The author (*6*) has reported upon this same system of air washing as it was used in connection with similar studies with wheat kernels infected with *P. graminis tritici*. Every precaution was taken to guard against any possible infection from outside. The room was made tight by using roofing cement to seal all cracks and openings in the sides and roof.

The first experiment was started on December 24, 1918. The seed used was for the most part from varieties grown in the rust nursery at Corvallis. It consisted of hand-picked kernels, all of which showed unmistakable evidence of stripe-rust infection. The varieties used were Hansia Brooch (C. I. No. 4690), Popatia Nadiad (C. I. No. 4696), Talimka (C. I. No. 2495), Baart (C. I. No. 1697), and Chul (C. I. No. 2406). In this, as in all other experiments of this series, temperature and humidity records were kept and it was found possible to maintain the temperature and relative humidity within the normal limits of plant growth. Flats filled with about 5 inches of sandy-loam soil were used for growing the plants. In all, 1,465 wheat plants were grown to maturity in this experiment, and no rust appeared on any of these plants at any time.

The second experiment was started on November 20, 1919. Wheat kernels were picked in the same manner as for the first experiment and the same methods were followed. Chul wheat (C. I. No. 2406) was used for the entire lot of plants in this experiment. The total number of plants grown was 2,470. No rust appeared at any time on any of the plants grown from this rust-infected seed and the experiment was discontinued when the wheat was mature.

In addition to the two experiments outlined above, 786 plants were grown from rusted seed in one of the rooms in the greenhouse without any special attempt to isolate the room to keep outside infection from the plants. These were grown to maturity during the winter months without the development of rust on any of them.

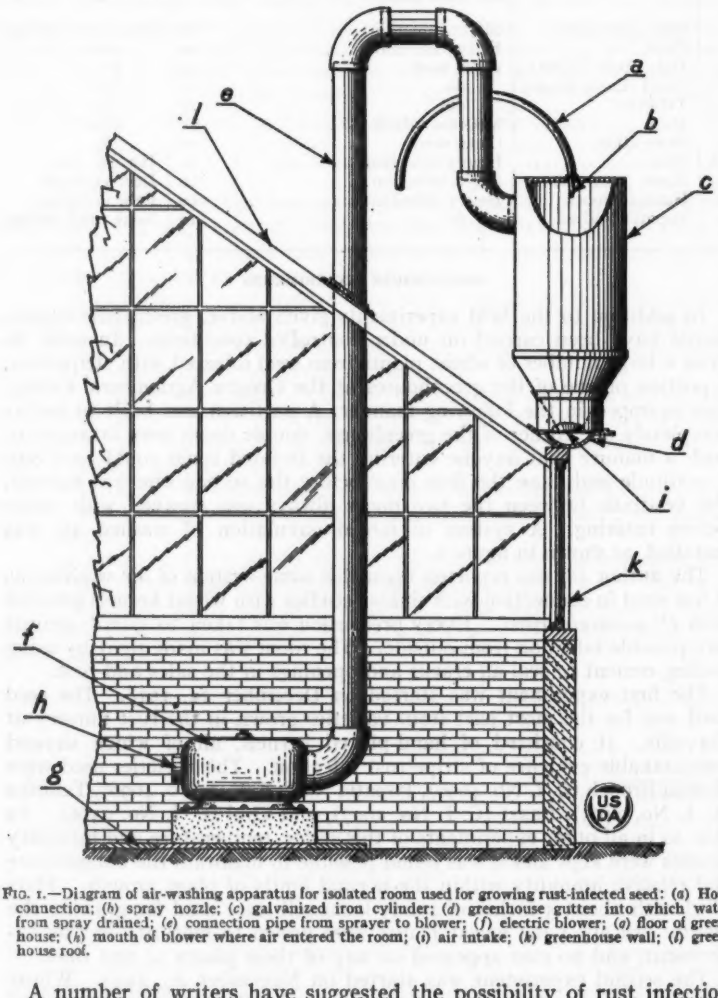

FIG. 1.—Diagram of air-washing apparatus for isolated room used for growing rust-infected seed: (*a*) Hose connection; (*b*) spray nozzle; (*c*) galvanized iron cylinder; (*d*) greenhouse gutter into which water from spray drained; (*e*) connection pipe from sprayer to blower; (*f*) electric blower; (*g*) floor of greenhouse; (*h*) mouth of blower where air entered the room; (*i*) air intake; (*k*) greenhouse wall; (*l*) greenhouse roof.

A number of writers have suggested the possibility of rust infection taking place from urediniospores on the surface of the seed or in the soil. To test this possibility, several flats were sown with wheat seed that had been covered with viable urediniospores of *P. glumarum tritici*. Chul wheat (C. I. No. 2406) was used for this experiment. About 300 plants were grown to maturity from seed thus treated. No rust infection appeared upon any of them at any time.

SUMMARY

(1) Observations show that stripe rust, caused by *Puccinia glumarum* (Schm.) Erikss. and Henn., may overwinter on the Pacific coast, both as mycelium and as urediniospores, on wheat and wild grasses. It was possible to collect viable urediniospores during every month from September, 1917, to July, 1918, at Corvallis, Oreg.

(2) Although the experiments which have been carried on are not absolutely conclusive, there is good evidence that the mycelium of stripe rust may overwinter at Moscow, Idaho, on *Hordeum jubatum* and *Bromus marginatus*.

(3) It has been found that the mycelium of stripe rust passes the dry summer months on the Pacific coast as dormant mycelium in the leaves of wild grasses. It is suggested that the climatic conditions which may prevail during the summer and fall of a given year determine whether or not an epidemic of stripe rust may develop upon winter wheat. The quantity of oversummering uredinia would be an important consideration in producing an epidemic on fall-sown wheat.

(4) Germination tests with urediniospores of stripe rust show that when the leaves of the infected host are kept in herbarium packets at ordinary room temperature the spores may remain viable at least 58 days. Urediniospores on leaves of wheat kept in open vials in a desiccator gave a slight percentage of germination at the end of 63 days. Urediniospores taken from wheat leaves, placed on glass slides and kept in a protected place in the laboratory, gave a trace of germination in 23 days.

(5) Urediniospores of *P. glumarum tritici* did not prove to be so resistant to desiccation as urediniospores of *P. graminis avenae*, *P. triticina*, or *P. holcini*.

(6) It has been shown that infection by urediniospores of stripe rust can not take place before the primary leaf of the wheat seedling has begun to expand.

(7) The period of incubation for the uredinial stage of stripe rust has been found to be 12 to 13 days under conditions favorable for infection. Low temperature and lack of sunlight may materially lengthen this period.

(8) The uredinia and telia of stripe rust occur commonly upon kernels of certain varieties of wheat. As high as 60 per cent of the kernels of certain varieties grown in a rust nursery were infected. Over 35 per cent of infected kernels have been found in wheat grown under ordinary field conditions.

(9) The germination of seed infected with stripe rust was only 50 per cent of that of the uninfected seed from the same seed lots.

(10) Forty rows, each 1 rod long, of rust-infected seed of several varieties of wheat were sown on October 10, 1917. No stripe rust developed on the plants from this seed before the next spring. Plants from clean seed sown at the same time became infected the next spring at about the same date as the plants from the infected seed.

(11) More than 4,700 plants were grown from rust-infected wheat seed in a specially constructed room in the pathological greenhouse at the Oregon Agricultural College, and no rust appeared upon any of them at any time.

(12) No infection appeared upon plants grown from wheat seed which had been covered with viable urediniospores of stripe rust before sowing.

(13) The results of the experiments here reported indicated that stripe rust can easily overwinter in the uredinial stage on the Pacific coast and

in the intermountain regions of the West. They further indicate that *P. glumarum tritici* is not transmitted from one wheat crop to the next by means of infected seed wheat.

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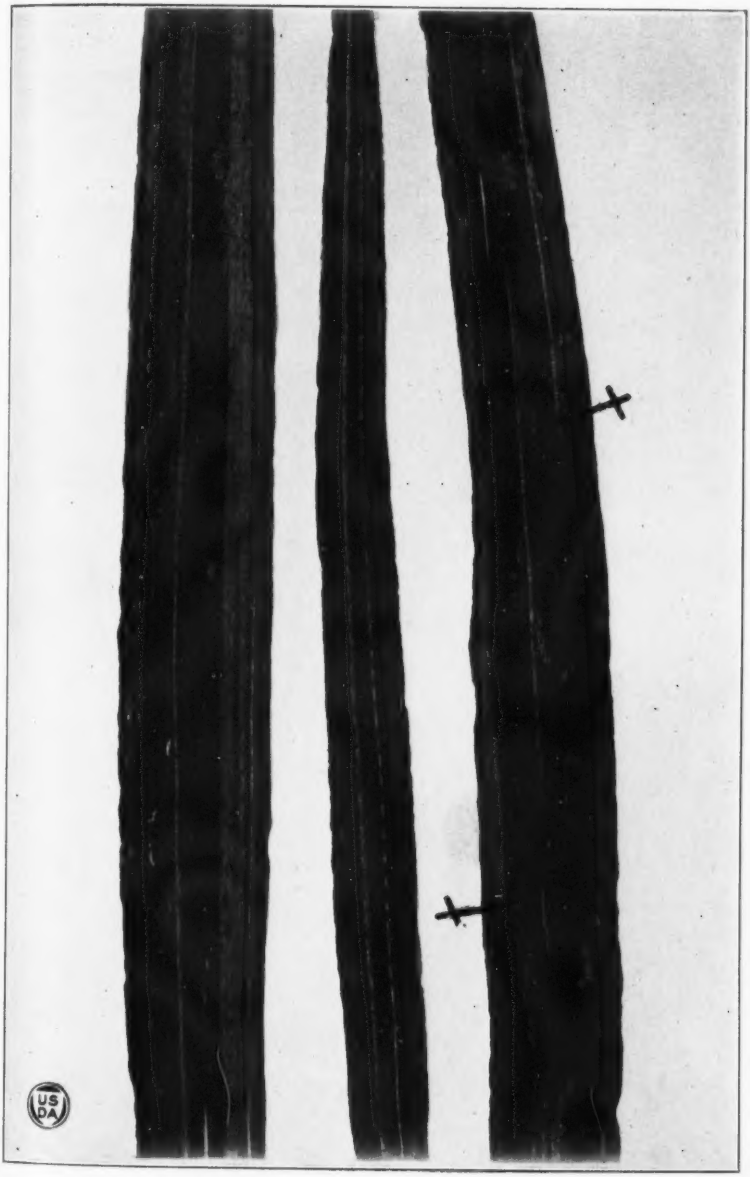
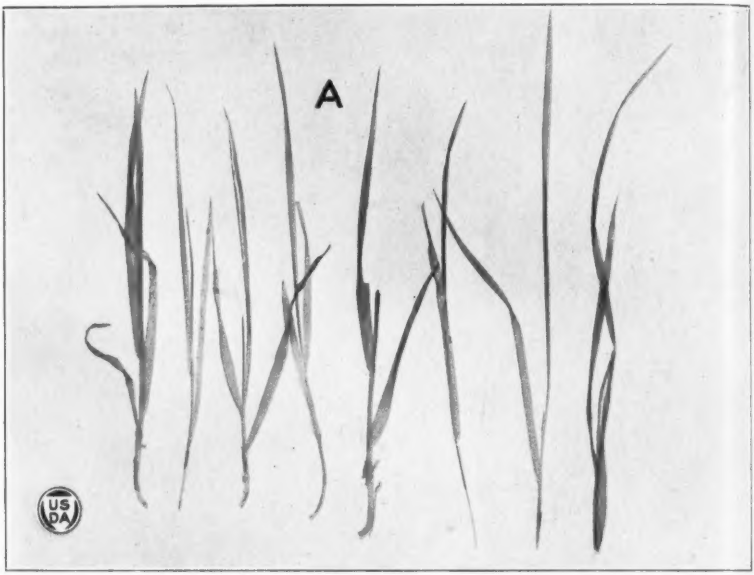
PLATE 1

Leaves of *Elymus glaucus* infected with *Puccinia glumarum*. These leaves were collected on September 20. Note telia on the stripes. A few uredinia are beginning to break through at x. This illustrates the method of oversummering on the host in the Pacific Coast region.

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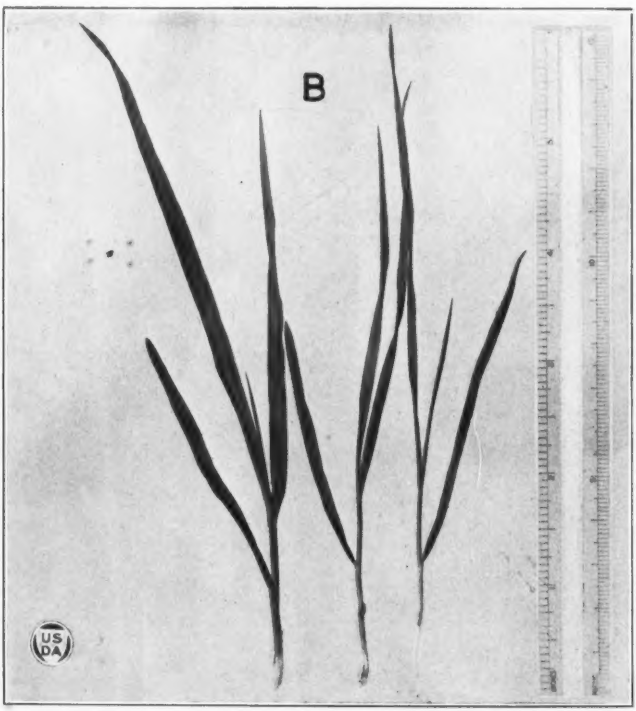
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PLATE 2

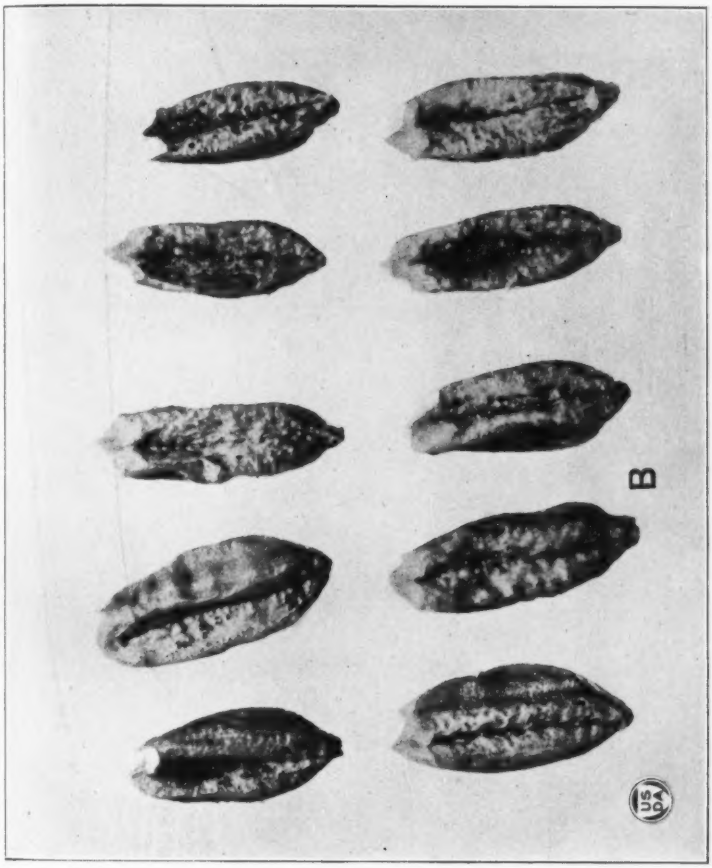
A.—Uredinia of stripe rust on seedlings of *Hordeum nodosum*. These plants became infected from older plants around which they were growing.

B.—Uredinia of stripe rust on seedlings of wheat which grew near older plants which were heavily infected. Seed from the same lot sown some distance from any infected plants produced seedlings free from the disease.

PLATE 3

A.—Two heads of Chul wheat grown in the greenhouse. The one on the right was inoculated with urediniospores of *P. glumarum tritici*. The one on the left was not inoculated. Infection just as severe has been noted to occur under natural conditions in the field.

B.—More or less shrunken kernels of Dale (Dale Gloria) club wheat showing both telia and uredinia of *P. glumarum tritici* (much enlarged).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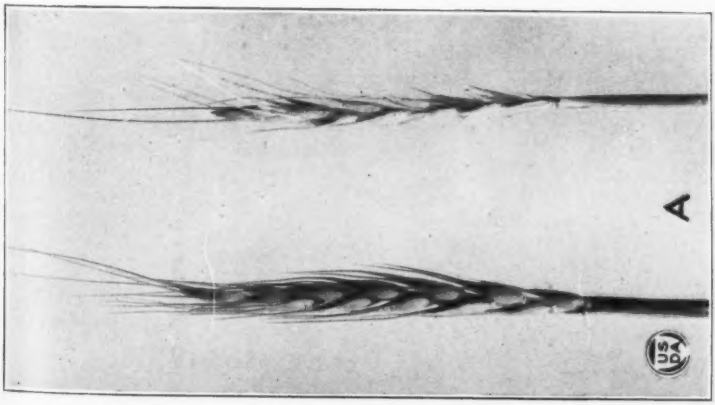
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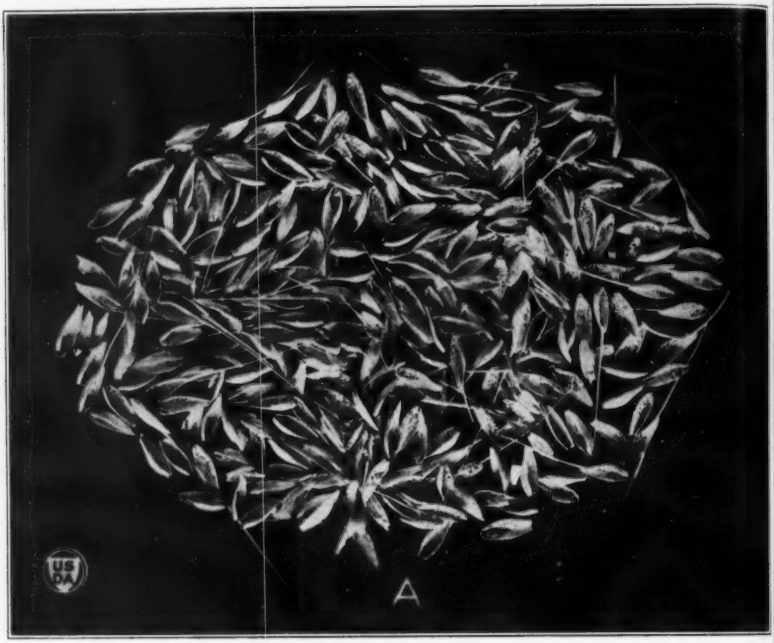
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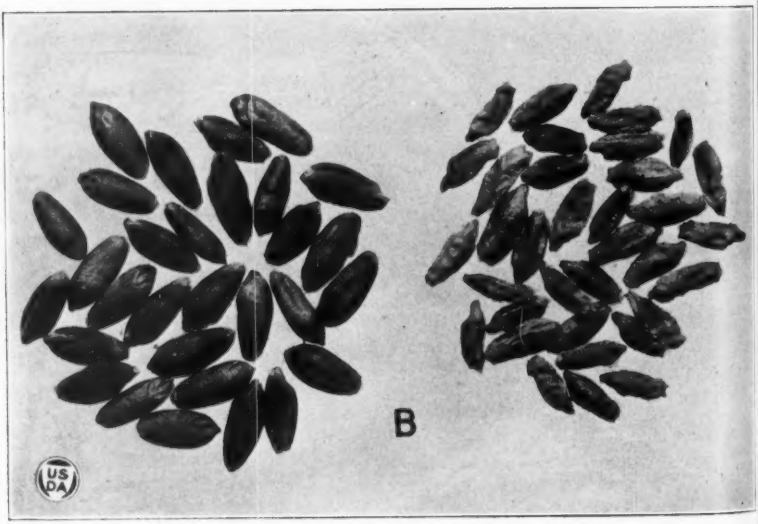
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PLATE 4

A.—Telia and uredinia of *P. glumarum tritici* on glumes of Chul wheat. This chaff was secured from straw after threshing. (Enlarged.)

B.—Two lots of Chul wheat. Those at the right are infected with stripe rust. Those at the left are healthy kernels from the same sample. (Enlarged.)

INFLUENCE OF SOME NITROGENOUS FERTILIZERS ON THE DEVELOPMENT OF CHLOROSIS IN RICE [1]

By L. G. WILLIS, *Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

DEFICIENCY OF IRON AS A CAUSE OF CHLOROSIS

The chlorosis affecting certain species of green plants when they are grown on calcareous soils has been extensively studied by Gile (*6*)[2] and Gile and Carrero (*9*), who carried on the greater part of their experimental work with rice and with pineapples. They offered strong evidence to support their opinion that such chlorosis is caused primarily by the precipitation of iron in the soil by calcium carbonate and the consequent inability of the plant to obtain a sufficient supply of iron for the development of chlorophyll.

Mazé, Ruot, and Lemoigne (*16*) have recorded that in some of their cultures even the small quantity of 0.2 per cent of calcium carbonate was sufficient to render certain species of plants chlorotic by depriving them of enough iron for the formation of chlorophyll.

FACTORS GOVERNING THE AVAILABILITY OF IRON

REACTION OF THE CULTURE MEDIUM

Gile and Carrero (*8*) further showed that in some solution cultures of neutral or alkaline reaction rice became chlorotic, and that such condition was attributable to precipitation of iron as phosphate and hydroxid. In their more acid solution, which had a reaction equivalent to a P_H of 3.1, these investigators noted an apparently higher degree of availability of all forms of iron used. In all these solutions nitrogen was supplied in the form of nitrates.

Working more recently with wheat in sand culture with nitrogen furnished in the form of calcium nitrate, McCall and Haag (*18*) have shown that solutions having P_H values ranging from 4.02 to 7.00 produced chlorotic plants, the color of which was not restored by the addition of ferric nitrate to the culture. The addition of very small quantities of sulphuric acid, however, resulted in marked improvement in the color of the plants, which became normal green at harvest time. These investigators were of the opinion that the indirect evidence supported the assumption that chlorosis in their less acid cultures was due to lack of available iron or to faulty metabolism resulting from immobility of iron in the plant.

SOLUBLE PHOSPHATES

Factors in addition to the reaction of the culture medium are known to influence the development of chlorosis. Crone (*2*) advanced the hypothesis that soluble phosphates in solution cultures caused chlorosis of plants. Takeuchi (*23*), on the other hand, using wheat as a culture plant, showed that the chlorosis observed by Crone was probably due to the precipitation of iron as a phosphate in the culture medium. Later, Sidorine (*22*), working with corn, offered evidence to show that chlorosis,

[1] Accepted for publication July 11, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 639-640.

due to lack of assimilable iron for the plant, may be induced either by an excess alkalinity or by the presence of large quantities of soluble phosphates in the nutrient. It is not improbable, however, that chlorosis that is due to precipitation of iron as phosphate could be obviated by proper adjustment of the reaction of the medium, though the degree of acidity necessary to accomplish this would be greater than it would be were the iron precipitated as hyroxid.

EXCRETION OF SOLVENTS FROM PLANT ROOTS

The reaction of the mass of a medium is not, however, necessarily the critical condition governing the availability of iron. Mazé, Ruot, and Lemoigne (*16*) attributed the resistance to chlorosis of some of their culture plants that were grown in alkaline media to the ability of the plants to excrete organic acids from their roots. These acids formed complex molecular compounds of iron that were soluble in the presence of calcium carbonate, and thus made available sufficient iron for the needs of the plant.

The solvent action of root excretion on insoluble matter which was in contact with roots was recognized by Takeuchi (*23*), who recommended the agitation of the precipitate in solution cultures to prevent chlorosis resulting from lack of iron. Mazé (*15*), too, stated that a marked influence was exerted on the plants in his solution when the roots reached the bottoms of the flasks or when the solutions were agitated and the precipitate settled on the plant roots. These observations indicate, as one significant difference between the reactions of plants grown in solution cultures and of those grown in soils, that, in the first instance, contact between the insoluble nutrients is not maintained and the solvent action of the root excretions is dissipated, and that, in the second instance, the influence of such material, being localized, exerts its maximum effect.

The excretion from plant roots of organic acids other than carbonic acid has, however, never been satisfactorily demonstrated. The results obtained by Kossowitsch (*14*) show that the elimination of carbon dioxid is apparently a normal function of the roots of many, if not of all, species of plants. It does not, therefore, seem probable that the variation in the susceptibility to chlorosis of different species of plants can be due under normal conditions to the excretion of a specific solvent.

It may be that variations in the quantities of carbon dioxid which are evolved from the roots of plants of different species determine the susceptibility of each plant to chlorosis when the plant is grown in less acid media; and as the source of this compound must be the carbon that is assimilated through the agency of chlorophyll, the activity of which in turn is dependent upon an adequate supply of iron, it is possible that in chlorotic plants the intensity of chlorosis depends, to a large extent, upon the balance existing between the potency of the agencies depressing the solubility of iron in the soil and the solvent power of the carbon dioxid that is eliminated from the roots.

EFFECT OF UNASSIMILATED RESIDUES OF FERTILIZER SALTS

It was shown by Kossowitsch (*13*) and by Mazé (*15*) that there might be a modification of the reaction of a culture as a result of plant growth and that, when ammonium sulphate was used as the source of nitrogen, the utilization of the basic radicle resulted in the release of the acidic radicle which, rejected by the plant at its roots, tended to produce an

acid condition of the medium. Similarly, when nitrates were used, the reaction of the medium was changed but in the opposite direction.

The effect of these residues on the insoluble nutrients in the soil had been demonstrated by Shulov (*21*), who found that when sulphate of ammonia was used as a source of nitrogen the insoluble phosphates in the soil were rendered more available to plants than when nitrogen was supplied as sodium nitrate.

Mazé, Ruot, and Lemoigne (*17*), too, suggested that the chlorosis, which developed in corn with which they experimented, was caused by the precipitation of iron by calcium carbonate that was liberated in the culture solution when the plant assimilated the acidic radicle of a calcium salt.

More recently, Jones and Shive (*10*) have shown that, in solution cultures of wheat plants, the availability of ferric phosphate, as indicated by the susceptibility of the plants to chlorosis, was governed by the degree of acidity of the medium which increased or decreased as a result of plant growth according to whether ammonium sulphate or calcium nitrate was used as a source of nitrogen.

It is probably true that in a solution culture in which there is no close contact between the plant roots and the precipitated material the reaction of the entire medium must be sufficiently acid to render the iron soluble in order to prevent chlorosis, and that the hydrogen-ion concentration at the threshold of chlorosis is the critical reaction governing the availability of the form of iron in that particular culture. In the denser media, however, in which the roots are in contact with insoluble material and in which circulation of the nutrient solution is retarded and sedimentation of precipitated matter is prevented, there may be, as a consequence of plant growth and the rejection by the plant of nonassimilable compounds, a very significant modification of the composition and reaction of the nutrient, the extent and intensity of which it is not easy to determine. Under such conditions it is evident that only that part of the culture which is in contact with the plant roots is the true medium in which the plant grows, and that the reaction of the unassimilated residue of the nutrient salts may be of more significance than is the reaction of the mass of the medium.

EFFICIENCY OF NITRATES AND AMMONIUM SALTS AS NUTRIENTS FOR RICE

It has long been known that rice (*Oryza sativa*) differs from the majority of other economic plants in that it does not seem adapted to the utilization of nitrate nitrogen as a fertilizer when it is grown under swamp conditions. Ammonium salts, on the other hand, have generally been found to serve as a suitable source of nitrogen, but Daikuhara and Imaseki (*3*) have noted that the difference between the efficiencies of the two forms of nitrogen disappears when rice is grown on soil that is not submerged.

Many attempts have been made to explain this characteristic preference of the rice plant for ammonium nitrogen. Nagaoka (*19*) suggested that three factors were involved in interfering with the utilization of the nitrates: (1) Loss of nitrogen by denitrification; (2) the formation of poisonous nitrites in the process of denitrification; and (3) the absence in the plant of sufficient sugar to transform the nitric nitrogen absorbed into protein.

Daikuhara and Imaseki (*3*) showed that the last theory was untenable when they proved by analysis that the sugar contents of paddy rice and of upland rice were approximately equal. Their results, however, indi-

cated that there was a great tendency to loss of nitrate nitrogen from leaching and denitrification in the submerged soil. They also observed that plants which were treated with nitrate became pale yellow in the early period of growth but that they recovered later. This was attributed to the physiological effect of poisonous nitrites which were formed by the reducing action of certain bacteria.

Kelley (*11*), too, found that rice made a weak yellow growth in pots when it was supplied with nitrates as a source of nitrogen, but that the plants usually assumed a deep green color as they neared maturity and apparently made normal growth thereafter. In one series of sand cultures to which sodium nitrate was added, however, it was observed that although repeated plantings were made, the seedlings in every instance stood for some days, turned yellow, and died. These results were considered to indicate that nitrates were unable to nourish the young seedlings properly, but Kelley also attributed injury causing the rice plants to turn yellow to the action of nitrites in the culture. Perciabosco and Rosso (*20*), however, showed that nitrites in culture solution were absorbed by rice without evidence of injury.

More recent work by Trelease and Paulino (*25*) shows that ammonium salts are more efficient sources of nitrogen for rice than are the nitrates. The results of Kellner (*12*) and of Espino (*4*), however, indicate that, while young rice plants require ammonium nitrogen for normal development, the older plants utilize the nitrates as well as they do ammonium salts. Gile and Carrero (*8*), on the other hand, have reported results obtained by the use of culture solutions in which nitrogen was supplied in the form of sodium and potassium nitrates; and while they did not claim that theirs was an optimum solution for rice, they stated that when the solution was sufficiently acid to insure the availability of enough iron, the rice made normal growth and was equal in size to exceptionally large field-grown plants. These investigators also observed that the yield of plants might be diminished by a deficiency of iron which was not great enough to affect the color of the leaves.

EXPERIMENTAL DATA

On account of the effect which the unassimilated residues of the different forms of nitrogen have on the reaction of plant culture media and also because of the relation existing between the reaction of the medium and the appearance of chlorosis, it seemed advisable to reconsider the question of the comparative efficiency of nitrates and ammonium salts for rice. It was therefore decided to make a study especially to determine the relationship between the two classes of compounds and the chlorosis due to the unavailability of iron for the plant.

For the earlier investigations the soil chosen was an acid red clay which, in a preliminary test, had been found to be very deficient in nitrogen and phosphorus. Plants that were grown on this soil were apparently immune to chlorosis regardless of the quantity of lime that was added to alter the reaction. In the response of plants on this soil to different forms of nitrates and ammonium salts no differences attributable to the form of nitrogen used were noted. The results, however, were rendered of doubtful value on account of an abnormality which was apparently the straighthead disease of rice that is described by Tisdale and Jenkins (*24*).

Further tests were made in which there was used an alkaline sandy soil from a river bank having the following composition:

Analysis of moisture-free soil

Constituents.	Per cent.
Material insoluble in HCl (sp. gr. 1.115)	68.73
Ferric oxid (Fe_2O_3)	9.43
Aluminic oxid (Al_2O_3)	12.32
Manganese oxid (MnO)	.17
Lime (CaO)	2.02
Magnesia (MgO)	1.50
Potash (K_2O)	.20
Soda (Na_2O)	.38
Phosphorus pentoxid (P_2O_5)	.07
Sulphur trioxid (SO_3)	Trace.
Carbon dioxid (CO_2)	.37
Nitrogen (N)	.034
Loss on ignition	5.47
Total	100.29

Preparatory to being used, the soil was put through a process to crush the lumps, and it was then passed through a screen (4 meshes to the linear inch). After being thoroughly mixed, 45 pounds of the air-dried material were then weighed into each of the required number of glazed earthenware pots having a 5-gallon capacity. No provision was made for drainage.

All treatments were added in solutions with sufficient water to saturate the soil, and the pots were placed on cars permitting of the carriage to a screened inclosure for exposure during fair weather and to a glass house at night and during rains.

Seed of the Wataribune variety was used for experimental plantings, a strain of which had been carefully selected for several generations for this purpose. Sufficient seed was planted to furnish about three times as many plants as were required, and the excess seedlings were removed so that the most thrifty plants would be well distributed in the pot. Plantings were made immediately after the treatments were added, and the soils were kept wet to the point of saturation until the plants were about 2 inches high. The soils were then flooded and kept covered to a depth of 1 or 2 inches until the plants were cut.

In a comparison made of several compounds to determine the best source of phosphorus for the soil, orthophosphoric acid (H_3PO_4) was found to give satisfactory results. This form was, therefore, used in all the experimental work to obviate any interference by inert material which might mask the effect of other treatments.

EXPERIMENT I.—COMPARISON OF AMMONIUM SULPHATE AND SODIUM NITRATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

Twenty-two pots of soil were given treatments of phosphoric acid and potassium sulphate to furnish each pot with 5 gm. of P_2O_5 and 2 gm. of K_2O. The nitrogen compounds were added in sufficient quantity to supply 1 and 2 gm. of nitrogen to the pots which received the lighter and the heavier treatments, respectively.

Of the pots which received each treatment, one-half were sprayed by means of an atomizer every two days with a 1 per cent solution of ferrous sulphate, preliminary tests having shown that ferrous sulphate was as efficient for spraying purposes as was ferric chlorid or ferric citrate. Five plants were selected from the seedlings to remain in each pot. Table I gives the plan of the experiment and the significant results obtained from it.

TABLE I.—*Chlorosis of rice on a calcareous soil as influenced by fertilization with sodium nitrate and ammonium sulphate*

SPRAYED WITH FERROUS SULPHATE

Pot No.	Nitrogen added as—		Analysis of solutions.								Weights of harvested plants.		Appearance of plants when cut.
			At planting.				At harvest.						
			Hydrogen-ion concentrations.	Parts per million of nitrogen present as—			Hydrogen-ion concentrations.	Parts per million of nitrogen present as—			Fresh.	Moisture-free.	
	$NaNO_3$	$(NH_4)_2SO_4$		NO_3	NO_2	NH_3		NO_3	NO_2	NH_3			
	Gm.	*Gm.*	P_H				P_H				*Gm.*	*Gm.*	
1	2		7.6	213	0.6	0.7	7.4	80	0.2	0.1	133	24.9	Yellow spotted with dark green.
2	2		7.8	288	.7	.7	8.1	80	.1	0	119	23.6	Do.
Average			7.7	251	.7	.7	7.8	80	.2	.1	126	24.3	
3		2	7.7	0	0	2.4	7.7	0	0	0	277	51.7	Dark green.
4		2	7.6	0	0	1.8	7.6	.6	0	.1	242	47.3	Do.
Average			7.7	0	0	2.1	7.7	.3	0	.1	260	49.5	
5	1		7.6	144	1.4	1.2	7.9	0	0	.1	110	23.8	Light green spotted with dark green.
6	1		7.6	163	1.4	.6	8.1	0	0	0	118	24.2	Do.
Average			7.6	154	1.4	.9	8.0	0	0	.1	114	24.0	
7		1	7.8	0	0	.3	8.1	0	.1	.1	(a)	(a)	
8		1	7.7	.2	0	1.5	7.9	0	.2	.1	171	34.6	Dark green.
Average			7.8	.1	0	.9	8.0	0	.2	.1	171	34.6	

NOT SPRAYED

NOT SPRAYED

9	2	.....	7.6	250	1.5	0	8.4	90	0	0	85	14.1	
10	2	.....	7.9	325	1.1	0	8.3	140	0	0	120	21.2	Yellow.
Average	.....	.....	7.8	288	1.3	0	8.4	115	0	0	103	17.7	
11	.....	2	7.8	0	0	.6	8.0	0	0	.1	320	59.5	
12	.....	2	7.9	.2	0	.4	8.0	0	0	0	247	47.7	Dark green.
Average	.....	.....	7.9	.1	0	.5	8.0	0	0	.1	284	53.6	
13	1	.....	7.7	150	1.3	0	7.9	0	0	0	128	25.3	
14	1	.....	7.8	125	1.5	0	7.9	0	0	.1	118	24.5	Light green.
Average	.....	.....	7.8	138	1.4	0	7.9	0	0	.1	123	24.9	
15	.....	1	7.9	0	0	1.2	7.9	0	0	.1	161	32.4	
16	.....	1	7.9	.1	.6	1.8	8.0	0	0	.1	145	30.6	Dark green.
Average	.....	.....	7.9	.1	.3	1.5	8.0	0	0	.1	153	31.5	
17	2	.....	7.9	225	1.2	.2	8.3	140	.3	1.0	(b)	(b)	
18	2	.....	7.8	250	1.2	0	8.3	240	.2	1.2	(b)	(b)	
Average	.....	.....	7.9	238	1.2	.1	8.3	190	.3	1.1	.....	.....	
19	.....	2	7.8	0	0	.5	8.3	.6	0	1.0	(b)	(b)	
20	.....	2	7.8	.2	.1	.6	8.4	1.2	0	1.6	(b)	(b)	
Average	.....	.....	7.8	.1	.1	.6	8.4	.9	0	1.3	.....	.....	

[a] Lost.
[b] Not planted.

The plants in this series of pots were cut while they were in the boot stage, it having been observed that those in soil cultures that had been rendered partly chlorotic by means of fertilizer treatment generally recovered their normal green color at about this period.

The results of this test show the influence on the development of chlorosis of the form of nitrogen used as a fertilizer and that the nitrate furnished was probably active in causing chlorosis, the effect being more marked on those pots receiving 2 gm. of nitrogen in that form than it was on those pots to which 1 gm. was applied. It would seem, therefore, that the calcareous soil was not the sole causative factor in similar cases of chlorosis, although this point has not been definitely established by the results obtained from the experiment.

That chlorosis was due to lack of sufficient iron for chlorophyll formation was evidenced by the development of a green color where the leaves were wet with the solution of ferrous sulphate. The nature of the leaf surface, however, was such that it was not possible to wet the entire area with the spray solution, and the plants therefore presented a mottled appearance. For this reason it is not certain whether the plants that were fertilized with sodium nitrate would have made a growth equal to that of the plants which received the ammonium sulphate had the spraying been more efficient as a remedy for chlorosis.

Observations made in this test do not show whether the plants which received sodium nitrate were chlorotic as a result of the form of nitrogen used or on account of the basic residue; whether the chlorosis, if caused by the basic residue, was due to the alkalinity of that residue or to the specific action of the sodium ion; nor whether there was a depression in plant growth additional to that caused by chlorosis.

A wide variation was observed in the size of the individual plants in the same pot and also in the tendency of plants of different sizes to recover from chlorosis. Apparently, absolute uniformity of cultural condition was not maintained in the experiment and the variations present governed the severity of the chlorosis.

Samples of the soil water for analysis were drawn from the mass of the soil by means of a pipette. The method of sampling was not ideal for the accurate determination of the composition of the nutrient solution, but it was chosen because it permitted the least disturbance of the plant roots. The determinations of hydrogen-ion concentration that were made by the colorimetric method of Clark and Lubs (*1*) are believed to be reasonably accurate, but the results showing the concentration of the different forms of nitrogen are only approximately correct.

The failure of the analysis to show the presence of appreciable quantities of ammonia is probably due to the absorption of that nutrient by the soil. Such removal of nitrogen from solution did not, it is thought, in any way interfere with the ability of the plant to absorb a sufficient amount for efficient utilization.

Evidence of the loss of some nitrogen through denitrification is shown by the decrease of nitrate nitrogen in the uncropped pots No. 33 and 35 during the period in which the plants were growing in the other pots. This loss, however, is thought to be of little significance in a comparison of the effects of the two forms of nitrogen.

The small quantity of nitrate nitrogen which accumulated in pots No. 34 and 36 as a result of the nitrification of ammonium sulphate may indicate that, as Fraps (*5*), Kelley (*11*), and Kellner (*12*), and others have shown, nitrification does not take place in submerged soils; or it

may indicate that denitrification processes were rapid enough to prevent the accumulation of considerable amounts of nitrate nitrogen. It is assumed, however, that in this experimental work the nitrification of ammonium sulphate was so greatly inhibited that the plants which were grown in pots treated with that compound derived the greater part of their nitrogen from the ammonium radicle. Nitrogen in the form of nitrite did not appear in significant quantities in any pot.

EXPERIMENT II.—A COMPARISON OF AMMONIUM SULPHATE AND AMMONIUM PHOSPHATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

No direct method having been devised to eliminate the effect of the basic residues of the nitrate fertilizers in this soil, in order to test the effect of nitrate nitrogen on the development of chlorosis it was planned to determine whether or not resistance to chlorosis was characteristic of plants grown with ammonium compounds.

Thirty-four pots were prepared in the same manner as were those in experiment I. Potassium sulphate (K_2SO_4) was applied in sufficient amounts to furnish 2 gm. of potash (K_2O) to each pot, and phosphoric acid (H_3PO_4) and ammonium sulphate [$(NH_4)_2SO_4$] were used as sources of phosphorus and nitrogen in one-half of the number of pots, while monoammonium phosphate ($NH_4H_2PO_4$) was used to supply the same nutrient elements in the other half. Nitrogen in the amount of 1.25 gm. was added to each pot, and phosphorus was furnished in the amounts shown in Table II. The ammonium phosphate treatments were supplemented with ammonium sulphate to maintain the uniform quantity of nitrogen per pot. Plantings were made as in the former experiment, and five plants were selected from the seedlings to remain in each pot. The spraying was conducted as in experiment I. The results are given in Table II.

TABLE II.—*Chlorosis of rice on calcareous soils as influenced by fertilization with ammonium phosphate and ammonium sulphate*

Pot No.	Nitrogen added as—		Phosphoric acid added as—		Yield of dry matter.	Hydrogen-ion concentration of soil water at harvest.	Number of plants.	Appearance of plants.
	$NH_4H_2PO_4$.	$(NH_4)_2SO_4$.	$NH_4H_2PO_4$.	H_3PO_4.				
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pu.*		
SPRAYED PLANTS CUT 42 DAYS AFTER PLANTING								
1	.....	1.25	.....	0.1	4.1	7.7	5	Green.
2	.....	1.25	.....	.5	6.0	7.7	5	Do.
3	.....	1.25	.....	1.0	9.6	7.7	5	Do.
4	.....	1.25	.....	2.5	9.7	7.7	5	Do.
5	0.02	1.23	0.1	.....	4.8	7.6	5	Yellow spotted with green.
6	.10	1.15	.5	.....	4.9	7.7	5	Do.
7	.20	1.05	1.0	.....	4.4	7.6	5	Do.
8	.50	.75	2.5	.....	2.4	7.6	5	Do.
UNSPRAYED PLANTS CUT 42 DAYS AFTER PLANTING								
9	.....	1.25	.....	0.1	2.9	7.5	5	Green.
10	.....	1.25	.....	.5	3.9	7.8	5	Do.
11	.....	1.25	.....	1.0	6.9	7.6	5	Do.
12	.....	1.25	.....	2.5	5.0	7.7	5	Do.
13	0.02	1.23	0.1	.....	1.3	7.7	5	Yellow.
14	.10	1.15	.5	.....	2.9	7.7	5	Do.
15	.20	1.05	1.0	.....	2.0	7.6	5	Do.
16	.50	.75	2.5	.....	.9	7.6	5	Do.
17	.....	1.25	.....	.....	4.0	7.7	5	Green.
SPRAYED PLANTS CUT 77 DAYS AFTER PLANTING								
18	.....	1.25	.....	0.1	60.1	7.2	5	Green.
19	.....	1.25	.....	.5	52.8	7.3	5	Do.
20	.....	1.25	.....	1.0	65.2	7.3	5	Do.
21	.....	1.25	.....	2.5	65.3	7.3	5	Do.
22	0.02	1.23	0.1	.....	51.0	7.3	5	Do.
23	.10	1.15	.5	.....	55.0	7.3	5	Striped.
24	.20	1.05	1.0	.....	60.6	7.3	5	Green.
25	.50	.75	2.5	.....	47.9	7.3	5	Do.
UNSPRAYED PLANTS CUT 77 DAYS AFTER PLANTING								
26	.....	1.25	.....	0.1	59.5	7.3	5	Green.
27	.....	1.25	.....	.5	46.0	7.4	5	Do.
28	.....	1.25	.....	1.0	53.5	7.4	5	Do.
29	.....	1.25	.....	2.5	57.5	7.5	5	Do.
30	0.02	1.23	0.1	.....	33.6	7.4	5	Yellow.
31	.10	1.15	.5	.....	27.9	7.3	5	Do.
32	.20	1.05	1.0	.....	9.5	7.7	1	Do.
33	.50	.75	2.5	.....	4.3	7.7	2	Do.
34	.....	1.25	.....	.....	54.6	7.5	5	Green.

In the original plan this test was to be conducted so that results could be obtained in duplicate, but 42 days after they were sown the plants under the influence of the different treatments showed such marked differences that it was deemed advisable to cut one-half the series in order that any tendency toward recovery might not deprive the writers of data showing the effect on plant growth of extensive chlorosis. At the time of the first cutting the plants in both pots in which the treatments had been duplicated were fairly uniform in size and appearance, so the probable error of the results obtained from the first cutting is small.

The weights of the plants that were cut when they were in bloom 72 days after planting are only approximately representative of the effect of the treatment because of the extreme variability in the size of the plants in many of the pots. In general, the variation was greatest in those pots showing the most severe cases of chlorosis, and it was apparently due to the unexplained difference in the tendency of individual plants toward recovery and normal growth. In pots No. 30 and 32 the chlorotic condition was so severe that some of the plants became diseased at the bud before they made any appreciable growth. The yields recorded for these pots represent only those plants which survived and made considerable growth after recovery.

As was true in experiment I, the effect of spraying with ferrous sulphate was only partly successful in overcoming chlorosis on account of the nature of the leaf surface. That either the iron applied was diffused throughout the leaf or that the effect of the partial development of green color was to aid the plant toward natural recovery was apparent soon after the first series of plants was cut when the spotted surface of the leaves of the remaining sprayed plants developed a striped appearance. By reference to Table II it may be seen that, regardless of the difference in fertilizer treatments, the plants that were sprayed were quite uniform in appearance, although the plants grown with ammonium phosphate were not equal in weight to those grown with ammonium sulphate.

The results obtained from experiment II show that the conditions causing the development of chlorosis were not necessarily induced by the presence of nitric nitrogen; nor were they traceable to the presence of an unassimilable basic radicle in the fertilizer salts used, in this case the indication being that chlorosis may have been caused by the precipitation of iron in the soil or in the plant by the phosphate ion liberated when the ammonium ion was assimilated at the more rapid rate.

The explanation is not definitely justified by the data at hand because it was not possible, owing to the small quantity of material available, to obtain analytical results showing the relative quantities of nitrogen and phosphorus assimilated. Moreover, it is not certain that in a calcareous soil ammonium phosphate would remain as such for any great length of time. The chlorosis which occurred in the presence of ammonium phosphate was much more severe than that which resulted from the use of sodium nitrate, but the plants grown with the former compound responded much better to spraying with ferrous sulphate and apparently tended toward a more complete recovery in the later stages of growth than did those grown with sodium nitrate.

EXPERIMENT III.—THE COMPARATIVE EFFICIENCY OF NITRATE AND AMMONIUM NITROGEN AS FERTILIZER FOR RICE WHEN THE INTERFERENCE OF CHLOROSIS IS DIMINISHED

In experiment I it was found (1) that the application of sodium nitrate to rice in a calcareous soil was attended by a chlorotic condition of the rice plant, and (2) that spraying with a solution of an iron salt was only partly effective in remedying the condition.

To determine, then, the comparative suitability of nitrate and ammonium nitrogen on this soil, an attempt was made to take advantage of certain individual rice plants which are less affected by the conditions causing chlorosis than are others, and also of a characteristic of the variety of rice used, the characteristic being that the rice tillers freely and produces culms and yields which are not closely dependent upon the maintenance of a definite number of plants of uniform size per unit area.

Eighteen pots were prepared and treatments were added in the same manner as they were in the previous tests, 2 gm. of nitrogen being furnished each pot in the forms of calcium nitrate, and ammonium sulphate. Calcium nitrate was chosen in preference to sodium nitrate to minimize, if possible, the intensity of the effect of the basic ion. Ammonium nitrate was used as a physiologically neutral form of nitrogen intermediate between the nitrates with basic residues and ammonium sulphate with acidic residues.

Potassium sulphate in sufficient quantities to furnish 2 gm. K_2O and phosphoric acid in quantities to supply 4 gm. P_2O_5 were added to each pot. Nitrogen treatments were replicated in six pots so that cuttings might be made at different stages of plant growth. It was planned to make the first cutting of plants representing each treatment at about the time that the plants which had received calcium nitrate were showing evidence of recovery from chlorosis, selecting for samples of the rice grown with calcium nitrate the plants in those pots in which chlorosis was most severe, so that those which were least affected and which might make the quickest recovery could be allowed to mature to afford a better comparison of the yields as governed by the efficiency of the different forms of nitrogen.

The second cutting was made when the heads were beginning to emerge from the boot, at which time all the plants in the test showed a remarkable uniformity in degree of maturity.

Seeding was made as in the other experiments. Ten seedlings were allowed to remain in each pot until the pots which had received calcium nitrate could be arranged in the order of their intensity of chlorosis. Two pots representing each treatment were then selected to be held for the first cutting, 10 plants being allowed to remain in each pot, and 4 seedlings were removed from each of the other six pots. Table III gives the results of the experiment.

TABLE III.—*Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth*

TABLE III.—*Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth*

Pot No.	Form of nitrogen.	Age of plants when cut.	Original.				First stage.						Second stage.						Mature.						
			Analysis of soil water.				Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.		Appearance of plants.
			P_H.	P. p. m. nitrogen as—			P_H.	P. p. m. nitrogen as—					P_H.	P. p. m. nitrogen as—					P_H.	P. p. m. nitrogen as—					
				NO_3	NO_2	NH_3		NO_3	NO_2	NH_3				NO_3	NO_2	NH_3				NO_3	NO_2	NH_3	Straw.	Heads.	
		Days.									*Gm.*						*Gm.*						*Gm.*	*Gm.*	
1	$Ca(NO_3)_2$	40	7.4	240	0.1	0	7.9	32	0	0	7.1	Small; chlorotic.	7.9	3.5	0.1	0.7									
2	...do	40	7.4	216	.2	0	7.9	100	0.1	0	6.1	...do	7.9	2.5	.1	0									
3	...do	40	7.5	216	.1	0	8.0	40	.1	0	2.2	...do	7.8	0	0	.4									
Average			7.4	224	.1	0	7.9	57	.1	0	5.1		7.9	2.0	.1	.4									
4	$Ca(NO_3)_2$	93	7.4	264	.1	0	7.8	20	0	0		Chlorotic	7.8	.5	.2	0	39.2	Green; chlorotic.							
5	...do	93	7.4	240	.2	0.1	7.9	80	0	0		...do	8.1	0	.2	0	52.2								
Average			7.4	252	.2	.1	7.9	50	0	0		...do	8.0	.3	.2	0	45.7								
6	$Ca(NO_3)_2$	114	7.4	264	.1	0	7.9	26	0	0		Large; chlorotic	7.5	0	0	.5		Normal	8.1	1.2			53	57	Normal.
7	...do	114	7.4	252	.1	0	7.9	34	0	0			7.6	0	0	.5		...do	7.8				58	59	Do.
8	...do	114	7.4	240	.1	0	7.7	30	0	0			7.8	.8	.2	.6		...do	8.0				36	54	Do.
Average			7.4	252	.1	0	7.8	30	0	0		...do	7.6	.3	.1	.5			8.0	.4			49	57	
9	NH_4NO_3	40	7.5	130	.2	0	8.1	0	0	0	14.3	Normal	7.6	0	.2	0									
10	...do	40	7.5	80	.1	.1	7.9	0	0	0	15.5	...do	7.9	2.5	.1	0									
Average			7.5	105	.2	.1	8.0	0	0	0	14.9		7.8	1.3	.2	0									
11	NH_4NO_3	93	7.5	120	.2	0	7.8	0	0	0		Normal	7.8	0	0	.3	62.2	Normal							
12	...do	93	7.5	130	.1	0	8.0	0	0	0		...do	8.2	0	0	.4	56.9	...do							
Average			7.5	125	.2	0	7.9	0	0	0			8.0	0	0	.4	59.6	...do							
13	NH_4NO_3	114	7.5	130	.1	0	7.8	0	0	0		Normal	7.7	0	.1	.4		Normal	8.1	.5			59	61	Normal.
14	...do	114	7.5	140	.1	.2	7.9	0	0	0		...do	7.8	0	0	.7		...do	7.8	.4			57	57	Do.
15	...do	114	7.5	120	.3	.1	7.9	0	0	0		...do	7.8	0	0	.7		...do	7.9	1.5			49	49	Do.
16	...do	114	7.5	130	.1	0	7.9	0	0	0		...do	7.8	0	0	.7		...do	8.0				38	38	Do.
Average			7.5	130	.2	.1	7.9	0	0	0			7.8	0	0	.6			8.0	.6			51	51	

TABLE III.—*Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth*—Continued

Pot No.	Form of nitrogen.	Age of plants when cut.	Original.				First stage.						Second stage.						Mature.						
			Analysis of soil water.				Analysis of soil water.						Analysis of soil water.						Analysis of soil water.						
			Ph.	P. p. m. nitrogen as—			Ph.	P. p. m. nitrogen as—			Yield of dry matter.	Appearance of plants.	Ph.	P. p. m. nitrogen as—			Yield of dry matter.	Appearance of plants.	Ph.	P. p. m. nitrogen as—			Yield of dry matter.		Appearance of plants.
				NO_3	NO_2	NH_3		NO_3	NO_2	NH_3				NO_3	NO_2	NH_3				NO_3	NO_2	NH_3	Straw.	Heads.	
		Days.						*Gm.*						*Gm.*						*Gm.*		*Gm.*		
17	$(NH_4)_2SO_4$	40	7.5	0.4	0	0	8.0	0	0	0	23.0	Normal	8.1	0	0.1	0								
18	do	40	7.5	1.4	.1	.1	8.2	0	0	0	23.2	do	7.7	7.0	0	0								
Average			7.5	.9	.1	.1	8.1	0	0	0	23.1		7.9	3.5	.1	0								
19	$(NH_4)_2SO_4$	93	7.4	0	0	0	8.1	0	0	0		Normal	7.9	0	0	0	67.5	Normal						
20	do	93	7.4	0	0	0	7.9	0	0	0		do	7.9	0	0	0	67.0	do						
Average			7.4	0	0	0	8.0	0	0	0			7.9	0	0	0	67.3							
21	$(NH_4)_2SO_4$	114	7.5	.4	0	0	8.0	0	0	0		Normal	7.9	0	0	.7		Normal	7.8	1.3		49	50	Normal.
22	do	114	7.5	1.6	.1	0	7.9	0	0	0		do	7.9	0	0	.7		do	7.7	1.0		57	59	Do.
23	do	114	7.5	0	.1	.2	7.9	0	0	0		do	7.5	0	0	.5		do	8.0	.8		55	56	Do.
24	do	114	7.5	0	0	0	7.9	0	0	0		do	7.5	0	0	.8		do	8.0			61	55	Do.
Average			7.5	.5	.1	.1	7.9	0	0	0			7.7	0		.7			7.9	.8		56	55	

In this test the rice that was fertilized with calcium nitrate was more or less chlorotic in all pots until after the first cutting was made. The plants that were furnished with ammonium nitrate were chlorotic for only the first few days of their growth. Plants that were fertilized with ammonium sulphate were not chlorotic at any stage of development.

There was a wide variation in the degree of chlorosis of the different plants which had been made chlorotic as a result of fertilizer treatment. It was therefore found advisable in the first cutting to include with the two pots treated with calcium nitrate a third pot in which the chlorosis was so severe that it was doubtful whether the plants would survive for a later cutting. At the time of the second cutting some of the plants in pots No. 4 and 5, which had been given calcium nitrate, had become green, and the others were only partly chlorotic. The plants from each of these pots were therefore divided according to their color into two samples to be weighed and analyzed.

As was found in experiment I, ammonia nitrogen was apparently almost completely absorbed by the soil very soon after the addition of the ammonium sulphate, and nitrites were not found in significant quantities at any time. This may have been due to the nonproduction of the latter form of nitrogen, to its rapid assimilation by the plants, or to conversion to other forms of such amounts as were produced.

The concentration of nitrates in the soil water had decreased considerably at the time of the first cutting, and when the second samples were taken the waters were nearly free of nitrates—a phenomenon which was coincident with the recovery of a majority of the plants from chlorosis and which may have been indirectly a contributory factor influencing recovery.

In regard to the average yield of mature heads per pot, the pots which were treated with calcium nitrate were practically equal to those which received ammonium sulphate, while the yield from the pots to which ammonium nitrate had been added was somewhat lower than that made by the others. The wide differences in yields from individual pots that were treated with ammonium nitrate may indicate, however, that the yield was influenced by some detrimental factors which did not cause other visible symptoms of injury; and it is possible that the relative inefficiency of the form of nitrogen used was not the cause of the low average yield.

The basis of selection of the pots in which the soil had been treated with calcium nitrate and from which the plants were to be harvested at maturity is open to criticism. It is thought, however, that the three pots in which the plants made rapid and complete recovery from chlorosis are comparable with the same number of pots of any other treatment, and that they are, for purposes of determining the influence of the fertilizer on the yield, more truly representative of the treatment than would have been an average which included some chlorotic plants. With this point in view it is evident that, under the conditions of this experiment, the nitrates, as represented by calcium nitrate, served for the nutrition of the rice plant as suitably as did ammonium sulphate when the factors causing chlorosis were inoperative.

ANALYTICAL DATA

As to the cause of chlorosis in the series of pots which received calcium nitrate, some interesting analytical data showing some of the differences between plants of the first and second cutting in experiment III and between green and chlorotic plants of the same cutting are given in Table IV.

TABLE IV.—*Composition of rice plants as influenced by nitrogenous fertilizers*

40 DAYS AFTER PLANTING

Pot No.	Dry matter per pot.	Increment dry matter per pot.	Nitrogen.	Nitrogen in increment.	Iron.	Iron in increment.	Ash.	Ash in increment.	Silica.	Silica in increment.	Silica-free ash.	Silica-free ash in increment.	Iron in ash.	Iron in increment of ash.	Iron in silica-free ash.	Iron in increment of silica-free ash.
	Gm.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	7.1		3.54		0.0223		19.05		11.78		7.27		0.1171		0.3067	
2	6.1		3.59		.0175		21.10		13.36		7.74		.0829		.2262	
3	2.2				.0333		26.72		19.84		6.88		.1246		.4840	
Average	5.1		3.56		.0220		20.96		13.55		7.40		.1049		.2973	
9	14.3		3.33		.0294		17.48		9.85		7.63		.1680		.3853	
10	15.5		3.38		.0342		17.45		9.77		7.68		.1960		.4453	
Average	14.9		3.36		.0319		17.46		9.81		7.66		.1827		.4162	
17	23.0		2.90		.0331		17.13		8.61		8.52		.1932		.3885	
18	23.2		3.09		.0345		17.05		9.00		8.05		.2023		.4286	
Average	23.1		3.00		.0338		17.09		8.81		8.28		.1978		.4082	

93 DAYS AFTER PLANTING

4	[a] 28.4		0.81		0.0190		14.43		8.74		5.69		0.1317		0.3339	
4	[b] 10.8		.81		.0184		18.68		13.10		5.58		.0987		.3297	
5	[a] 48.8		.86		.0161		13.00		7.67		5.33		.1238		.3021	
5	[b] 3.4		.95		.0208		22.57		15.63		6.94		.0922		.2997	
Average	45.7	40.6	.84	0.36	.0174	0.0169	14.47	13.66	8.94	8.36	5.53	5.30	.1202	0.1237	.3146	0.3190
11	62.2		.79		.0128		14.93		10.26		4.67		.0857		.2941	
12	48.6		.93		.0110		15.14		10.63		4.51		.0726		.2439	
Average	55.4	40.6	.85	[c]	.0120	.0047	15.02	14.09	10.42	10.62	4.60	3.47	.0799	.0334	.2609	.1356
19	67.5		.90		.0138		15.02		10.17		4.85		.0919		.2846	
20	67.7		.84		.0127		14.71		10.03		4.68		.0863		.2714	
Average	67.6	44.5	.87	[c]	.0132	.0025	14.86	13.70	10.10	10.77	4.76	2.93	.0888	.0182	.2773	.0854

[a] Green plants. [b] Chlorotic plants. [c] Loss.

The samples analyzed represent the entire plant above ground. The dry matter produced under the influence of each treatment was found to vary considerably, especially in those pots which received calcium nitrate, and the results of the analyses are therefore weighted in proportion to the amount of dry matter produced in the respective pot for the calculation of the average.

ANALYTICAL METHODS

The analytical methods used were essentially those prescribed by the Association of Official Agricultural Chemists.[3]

Nitrogen was determined by the Gunning method, sodium sulphate being used in place of potassium sulphate. In order to determine the ash and its constituents, the material was charred over a low flame, the sample was weighed, leached with dilute hydrochloric acid, and then filtered and washed on a tarred Gooch crucible having a pad of ash-free filter paper. The insoluble residue consisting of silica and carbon was dried and weighed and the carbon was burned off. The ash percentages were calculated from the weight of the original incinerated material by subtracting therefrom the loss of weight due to the ignition of the carbon. Carbon dioxid in the ash was not determined on account of the small quantity of material available. The quantity of silica reported is the sum of the weights of insoluble silica and of that in the hydrochloric acid extract which was obtained by evaporation and dehydration. Iron was determined by permanganate titration after it was reduced with zinc and by the Reinhardt method. The results obtained by each method were in agreement.

DISCUSSION OF RESULTS OF ANALYSIS

Table IV discloses some results which may have a positive significance in showing the influences causing the chlorosis in experiment III. Table III shows that the nitrates had practically disappeared from the soil water at the time of the second cutting, and Table IV shows that a marked decrease in the percentage of nitrogen in the plant occurred during the period between the first and second cutting, those plants which had received nitrogen as calcium nitrate having gained 0.36 per cent of nitrogen in the increment of dry matter, while the plants in the other two series showed a loss of total nitrogen.

If, therefore, the production of basic residues by the assimilation of the nitric fertilizer salts produced in the soil a condition that prevented the absorption of iron, or in the plant a condition which prevented the utilization of iron for the development of chlorophyll, the influence must have been removed before the plants in experiment III were ready for the second cutting, and at a time that was approximately coincident with the recovery of the plant from chlorosis.

A comparison of the average results of the iron determinations indicates that, in those plants which received calcium nitrate, the first cutting of the plants had less iron in the dry matter, in the ash, and in the silica-free ash than was found in the same components of the green plants grown with the other treatments. In the second cutting the chlorotic plants had the higher content of iron in the dry matter; and while the

[3] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig., 1908. Reprinted 1912.

percentage of iron in the ash was higher in the green plants, this was largely due to a high proportion of silica in the ash of the chlorotic plants. The percentage of iron in the silica-free ash was about the same in the green and in the chlorotic plants.

Such observations would indicate that the chlorosis in these pots was not due to a deficiency of iron, but Gile and Ageton (7) explained a similar result in their work as possibly being due to the fact that the tissues of chlorotic plants represent an earlier period of growth than do the tissues of green plants of the same age and that they therefore require a greater quantity of iron to prevent chlorosis. It would not be impossible, however, for iron in a chlorotic plant, though present in sufficient quantity, to be rendered inactive for the development of chlorophyll by abnormal conditions in the plant.

It is shown that the green plants had an ash content which was less than that of the chlorotic plants, but the high ash content of the latter may have been due to the depression of carbon assimilation and to a consequent small quantity of carbonaceous matter in the plant. The high silica content of the chlorotic plants can best be explained in the same way, and variations in the amount of this constituent in the plant ash are apparently of no importance.

In regard to the silica-free ash, the plants of all treatments had a general uniformity of compositions at the same stage of growth but gave some evidence that the use of calcium nitrate as a fertilizer depressed the absorption of this constituent up to the time of the second cutting. It is hardly possible, however, that the silica-free ash content of the plant can be considered as indicative of the way in which nitrate fertilizers cause the development of chlorosis, because the analysis of plants in the second cutting from the nitrate pots showed that there was no consistent difference between the chlorotic and the green plants in this regard.

As was shown of the nitrogen content, the rate of absorption of the ash constituents, with the exception of silica, tended toward diminution as the plant passed through the stage in which it began to recover from the condition causing chlorosis. It does not appear, however, that the analytical results presented offer an evidence that variation in the ash content of the plant at different periods of growth has any bearing on chlorosis.

SUMMARY

The development of chlorosis of rice on calcareous soils may be governed by the nature of the nutrients supplied, those compounds represented by sodium nitrate, calcium nitrate, and ammonium phosphate, which, in themselves or by virtue of an unassimilable ion, are the cause of the precipitation of iron, being associated with chlorosis, while the plants which have been supplied with ammonium sulphate in which the unassimilable radicle may serve as a solvent for iron may be thrifty and of normal color.

It has not been shown by the experimental methods used whether the basic material in the soil or the basic residues of the nitrates were primarily the cause of the conditions determining the appearance of chlorosis, nor whether the acidic residue of ammonium sulphate served to prevent chlorosis in the calcareous soil. Inasmuch, however, as it was shown in the first experiment that the degree of chlorosis attending the use of sodium nitrate as a nutrient was in proportion to the quantity

of that salt furnished, it seems possible that the basic residues of the nitrates were primarily the cause of the chlorosis and that the alkalinity of the soil was a secondary factor.

The reaction of the soil in which the plant roots are in contact with the insoluble supply of iron is shown to be of less significance in determining the availability of iron for the plant than are the effects of the products rejected by the plant roots.

Chlorosis of rice grown on the soil used was especially severe in young plants, and plants which were not too seriously affected showed a tendency to recover as they approached maturity. This phenomenon of recovery was coincident with a decrease in the rate of absorption of nitrogenous compounds, a fact which substantiates the evidence that the nitrate fertilizers were causative of the chlorotic condition, although it is possible that an increase in the elimination of carbon dioxid from the roots had some influence in aiding the plant to recover its normal color by making more iron available in the soil.

The observation that nitrates are less suitable than ammonium salts for the fertilization of young rice plants may be based on the influence on the plants at the period of greatest absorption of nitrogen of the unassimilated residues of the fertilizer used rather than on the inferiority of nitric nitrogen as a nutrient. It seems probable also that the injury which has heretofore been ascribed to the toxic effect of the nitrites, derived from nitrates by reduction, was but a manifestation of chlorosis which was caused by the action of the basic residues of the nitrate salts used as nutrients.

The nitrogen of calcium nitrate may be suitable equally with that of ammonium sulphate in the physiological processes of the rice plant when it is used under cultural conditions in which the reaction of the unassimilated residue of the nitrate does not interfere with the absorption and utilization of iron.

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